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SYMPOSIUM: SOME BIOLOGICAL EFFECTS OF RADIATION FROM NUCLEAR DETONATIONS[1]

INTRODUCTION TO THE SYMPOSIUM

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This symposium was designed to make biologists acquainted with some of the effects of radiations from nuclear detonations on biological material exposed at 1953 field tests. There is no restriction on the publication of the biological data after it has been reviewed, but there are certain facts which for obvious reasons are excluded from the reports. Contributors cannot discuss specific detonations, locations of the test materials, nor distances from the detonations. They cannot compare one detonation with another. None of these restrictions is of importance, since, as will appear, approximate dose estimates are available. If there are certain uncertainties, such as the exact proportion of neutrons and gamma rays, it is necessary to recall that field studies under the conditions necessary at the nuclear detonations can never be done with the precision of laboratory studies. With these limitations I think it can be said that the biological program at the nuclear detonations made valuable contributions, not only to defense but to radiation biology.

The biological test program was developed with the encouragement and support of the Department of Defense and of the Atomic Energy Commission as a part of the general program of study of the effects of nuclear detonations. The studies were under the direction of the test organization of the Los Alamos Scientific Laboratory and could not have been carried out without their effective cooperation and support. The major portion of the program, apart from the genetics tests, was under the charge of a planning committee and was developed by members of the staff of Los Alamos, of the U. S. Naval Medical Research Institute, and of the U. S. Naval Radiological Defense Laboratory. The single contribution from members of this

[1]Symposium, arranged by Alexander Hollaender, Oak Ridge National Laboratory, held at the Joint Session of the American Society of Naturalists and the Genetics Society of America at the meeting of the American Association for the Advancement of Science, Boston, Massachusetts, December 28, 1953.

group to be presented by Dr. Carter in this symposium represents only a small portion of the much larger biological program which they have initiated, some of which has already been reported in classified contributions.

It was only recently that an extensive series of genetics tests was added to the studies at the nuclear detonations, but because this symposium is being presented before an audience whose main interests are in this area, most of the contributions are concerned with the genetic effects. It is proper to point out, however, that many more radiation geneticists were associated with the program than appear in this symposium. The largest number of such participants were from the Oak Ridge National Laboratory working with the effective cooperation of Dr. Hollaender, but significant contributions have been made by geneticists from at least ten universities or colleges throughout the country. The data from the other investigators have been made available to the speakers here, and some of it will be cited in the course of the program. The initial program of biological tests at an earlier test operation was under the general direction of Dr. G. R. Leroy. The genetics program for the more recent detonations was under the over-all direction of R. L. Corsbie, director of the Civil Effects Test Group, and Commander E. P. Cronkite was in charge of the biological test program. I was responsible for arranging the genetics tests and coordinating them at the detonations. The work of determining the dosages and especially the ratio of gamma rays and neutrons was carried out by another group in the test organization. In connection with this work the cooperation of C. W. Sheppard of Oak Ridge National Laboratory was invaluable.

The general characteristics of the radiations at nuclear detonations are well-known, and they have been described for a "Hiroshima-Nagasaki" type bomb in The Effects of Atomic Weapons (McGraw-Hill, 1950). To avoid repetition and to aid in clarification of the papers to follow, it may help if a brief statement is given of some of the more general problems which the biological tests might be expected to solve. The initial ionizing radiations from nuclear detonations of most concern for living organisms are gamma rays and fast neutrons. One of the first questions to be asked was whether there are unexpected biological effects of these high energy radiations which are not already known from previous laboratory experiments.

Since the changes produced by gamma rays on living tissues had already received much study, emphasis was placed on the biological effects of fast neutrons. Both neutrons and gamma rays produce their biological effects by ionizations in tissue cells. Fast neutrons in passing through a tissue form through their secondaries a much greater number of ionizations per unit length of path than do gamma rays or X-rays, that is, the secondaries have a higher specific ionization. At the momentary high intensity of nuclear detonations would they show significant differences in biological effects from the effects of the much lower intensity neutron flux from the cyclotron? In general, then, the question was: How does the biological effectiveness of fast neutrons from a nuclear device compare with that of the more familiar laboratory sources of gamma or X-rays?

All these problems required separation of these two kinds of radiations in exposures of the biological test objects to the nuclear detonations. There is no practical method of cutting off neutrons, but the gamma rays can be selectively filtered out by the use of lead shields of sufficient thickness. This method was the one used in the tests. The Los Alamos Test Organization designed and had manufactured a series of hemispherical lead shields seven inches thick with a chamber for biological specimens inside along with temperature control and aeration apparatus. The radiation inside is predominantly neutron radiation, although with some gamma ray contamination. It was the development of these lead shields and their proper placement which made possible all the biological tests reported here and many others recorded only in the classified reports. While many other individuals aided in the field work, and particularly the NRDL personnel, it was above all Dr. Robert Carter who placed the lead exposure chambers in the field so that the proper ranges of neutron dosage were available for the different biological materials in every detonation. This was of special importance for the genetics tests, since for each particular organism effective doses of radiation must be given at levels which include the LD 50 (lethal dose for 50 per cent) for the different organisms. These levels ran from about 100 rep (roentgens equivalent physical) to 50,000 rep or more, so the lead shields had to be put into functional positions over a wide range of distances, and they had to be reset for every detonation. That this was accomplished successfully indicates the effectiveness of the work of Dr. Carter and his associates from Los Alamos and NRDL.

The paper by Drs. Carter, Bond, and Cronkite will summarize the methods which they developed for measuring neutron doses by studies of exposed mice. Of these the reduction in thymus weight was the most sensitive biological dosimeter, and gave the data for a curve which by extrapolation made possible the location of the exposure chambers in the field with very little wastage after the first exploratory series. In addition, the biological effectiveness of neutrons from the detonations as compared with gamma rays was established for these somatic effects of radiation.

The genetic effects of neutrons from the detonations require separate extensive tests, since they are not identical with the somatic changes. For one thing, the organism as a whole may recover from single or even repeated exposures to low level radiation, yet the same radiation dosages will cause increases in mutation frequency which are permanent and cumulative. As for somatic effects, it is important to know if neutrons from nuclear detonations show similar genetic effects to those from neutron fluxes at lower intensities from cyclotrons or other neutron generators.

In addition, genetic effects are of different kinds, and each kind shows different degrees of response to the different kinds of radiation. There are the disruptive effects of radiations on chromosomes, resulting in breakage or other kinds of aberrations. These may appear in single chromosomal elements (chromatids) or in the doubled chromosome strand, each with a different frequency. Translocations and dominant lethals are also commonly

measured results of chromosomal damage. Then again, radiations produce an increase in gene mutations, which are usually thought of as changes in molecular configuration, or more strictly chemical changes in genes as contrasted with the gross effects like chromosome breaks. Finally, there are other more complex genetic changes which may be combinations of each of the kinds mentioned. The sex-linked lethals particularly appear to fall in this class. Each of these kinds of genetic change needed to be studied by exposures to the same series of radiations from nuclear detonations in order to secure convincing critical results.

The four reports on genetic effects in this symposium give representative results which will receive added confirmation when the reports of other investigators associated in this study appear. In order that they shall be understandable to geneticists less familiar with handling radiation data, it may be helpful to give some simple definitions which may be supplemented by such general reference material as the introductory section on "Radiation in Living Matter" by P. Morrison in Symposium on Radiobiology (Wiley, 1952) or "Biological Effects of Radiations" by H. J. Curtis in Biological and Medical Physics, vol. II (Academic, 1951). For biological effects, radiation exposure is most conveniently measured in terms of the roentgen (r), which is the amount of gamma (or X) radiation that produces in 0.00129 gram of dry air (1 cc at 0°C and 760 mm) ion pairs carrying a total of one electrostatic unit of charge of either sign. This amount dissipates about 83 ergs of energy per gram of dry air and about 93 ergs/gram in water. In considering the effects of two different sorts of radiations on biological material, the results must be given in comparable terms, namely, the amount of the particular radiation which will release 1 roentgen or 93 ergs per gram in the tissue being studied. This amount is the rep (Roentgen Equivalent Physical). It is therefore the amount of ionization produced in the particular tissue equivalent to that produced by one roentgen of gamma (or X) rays, or to state it in another way, the rep measures equal energy deposition in a tissue whatever the kind of radiation.

As already suggested, it might be expected that one rep of each different sort of radiation would produce a similar biological effect, but due to specific ionization, this is not necessarily so. It is not unreasonable that a greater concentration of ion pairs along one path should produce a greater biological effect than the same number of ions widely dispersed. This in fact is what has already been shown for neutrons as compared with X-rays. Thus the same biological effect, such as a given frequency of mutation, may be produced by 100 r of X or gamma rays but by much less than 100 rep, or the equivalent dose of neutrons. The measure of this difference in biological effectiveness of different radiations is the RBE (relative biological effectiveness), and it is the ratio of dose of X-rays/dose of neutrons required to produce an equal effect. This greater RBE of neutrons as compared with gamma rays was a major subject for investigation in the program being reported at this symposium. Because of the wide variation in values for RBE of neutrons in the literature (from 1 to 20), it was de-

cided that it was important to compare these RBE values for neutrons from the detonations with values from cyclotron or other laboratory neutron radiation. The recording of a wide range of values for genetic effects following neutron irradiation compared with X-rays and closely similar values for detonations and laboratory neutrons demonstrates the validity of the previous observations and indicates the importance of such a wide series of experiments made at the same time.

In addition to the participants in the present symposium the following took part in the genetics test program, and their contributions will form an essential part of the complete report of the studies: (K. C. Atwood, W. K. Baker, J. Kirby-Smith, E. F. Oakberg, D. Schwartz, and C. P. Swanson, all of the Oak Ridge National Laboratory; A. F. Blakeslee, and Jean M. Cummings, Smith College; J. W. Gowen, Iowa State College; P. T. Ives and H. T. Yost, Jr., Amherst College; J. B. Rowell, University of Minnesota; J. L. Spencer, University of Massachusetts; W. S. Stone, F. Clayton, E. Dudgeon, and M. L. Alexander, University of Texas; P. W. Whiting, and D. T. Ray, University of Pennsylvania; A. F. Yanders, Northwestern University.

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RADIOBIOLOGICAL STUDIES WITH TRADESCANTIA AT NUCLEAR TEST DETONATIONS[1]

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The flowering plant Tradescantia has been used at two nuclear test operations as an experimental organism for the biological effect of the ionizing radiations emitted. Blast and thermal effects of the test device have been excluded and are not considered in these experiments.

Previous to the earlier of the two operations, there were several uncertainties about what test radiation would do to living tissue. The unique circumstances of test radiation—which is emitted at very high intensity, consists of a mixture of gamma, β^-, neutrons, and α radiations, and is distributed over a very wide range of energies—were the reasons for the genuine uncertainties about how much tissue damage would be caused by test radiation. Some of these biological questions, at least previous to the earlier tests, were: (1) Would the test radiation cause any new or novel effects? Probably a negative answer would be given by most of the biologists concerned with these tests. (2) Would a given dose of test radiation and the same dose of radiation given in the laboratory cause equal effect? Expressing this in another way, could instruments which had been found reliable for measurement in the laboratory be accepted as reliable substitutes for biological measurement of test radiation effect? This amounts to asking if the relative biological efficiency of test radiations is the same as these radiations when they are delivered by the usual laboratory sources.

It should become apparent in the various test experiments to be described in this symposium that most of the facts and conclusions to be derived, even qualitative ones, depend almost entirely on a correlation between instrumental and biological measurements.

The intention was to estimate biologically the test radiation dose on the basis of chromosomal breakage in Tradescantia, and to compare this biological estimate with, among other things, the physical measurements made simultaneously. The method of biological estimate is simply described. The yield of chromosomal aberrations as a function of dose was derived from a series of laboratory control experiments with measured doses of radiation, designed to simulate as closely as possible the anticipated conditions of test exposure. When the yield of aberrations from the test irradiation had been measured, it was a simple matter to solve for the single unknown, test dose, on the basis of the values for aberration-dose relation from the control experiments: these are called "biologically estimated dose."

[1]Text of a talk given before a Symposium at Boston in December, 1953, sponsored by the American Society of Naturalists and the Genetics Society of America.

Statistical confidence intervals, including all errors from both the control *and* the nuclear test detonation data, can be assigned to these biological dose estimates. The data for the first operation alone are based on observation of over 19,000 chromosomal aberrations found in 17,000 cells and involve 56 different radiation doses. Only the final results of the Tradescantia experiments will be considered here. Most of the data from which they are derived have been published (Conger, 1954).

Fig. 1 is an example of one of the control experiments and shows the relation of aberration yield to dose of radiation. In this experiment, a control for the nuclear test gamma-ray exposures, irradiation was by hard X-rays given at high intensity. Fig. 2 is plotted from the data of another control experiment, and shows the increase of chromosomal-aberration yield with dose of fast neutrons from uranium fission. With fast neutrons, as can be seen, these particular aberrations increase linearly with dose, but with X or gamma rays they increase as a power of the dose. Considerations such as these make it important that for the test dose estimates the conditions in the test and the control irradiations (i.e., simulated test) be the same.

Tradescantia and R. E. Carter's mice (Carter *et al.*, 1954) were exposed to radiation from nuclear test devices in three different situations at the first operation, as follows: (1) inside airplanes flown through atomic clouds at different altitudes, (2) to gamma radiation (inside thin protective containers)

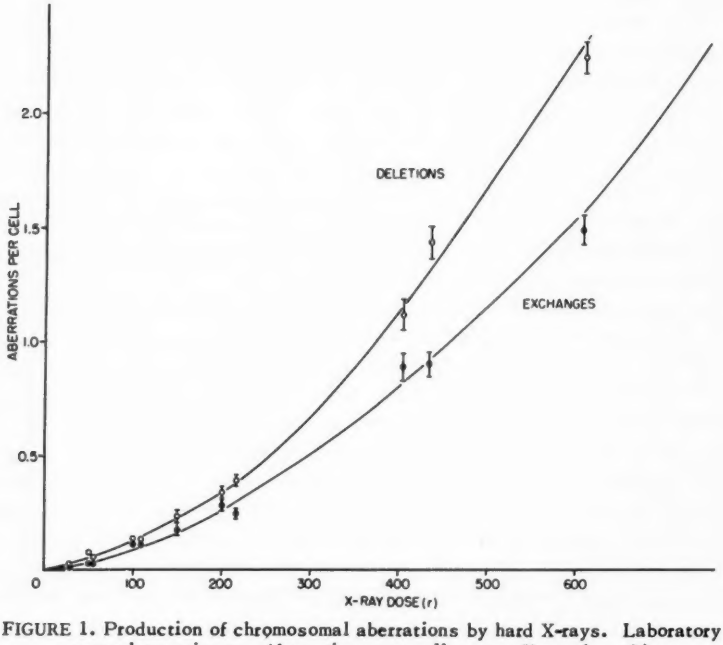

FIGURE 1. Production of chromosomal aberrations by hard X-rays. Laboratory control experiment. Aberrations per cell versus X-ray dose (r).

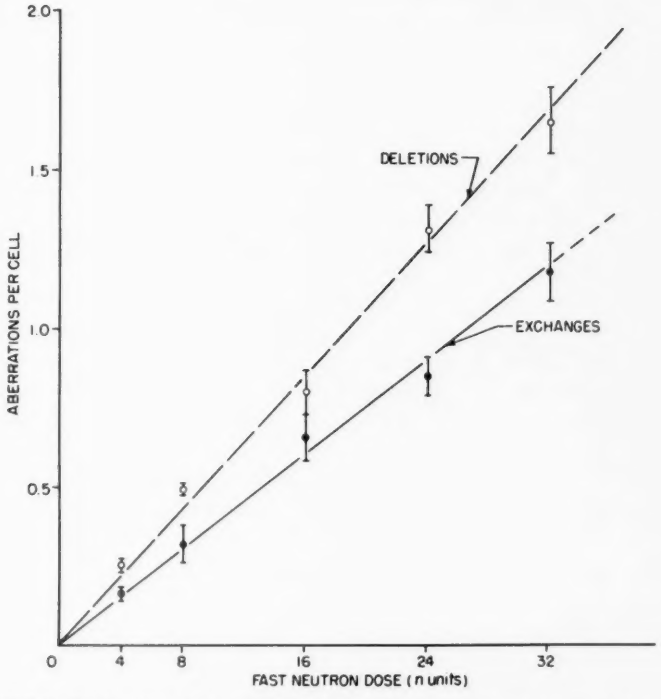

FIGURE 2. Production of chromosomal aberrations by fast neutrons. Laboratory control experiment. Aberrations per cell versus fast neutron dose (n units).

at various distances, along the ground, away from the nuclear test device, and (3) to mostly fast neutron radiation in special containers, at closer distances than the gamma-ray stations. On the second operation, only neutron exposures, in the same type of container, were made.

The Tradescantia data are presented in a form which shows the correlation between instrumentally measured dose and biologically estimated dose. In this way, a biological material is used as a dosimeter in which what is measured is the amount of some biological effect, in this case chromosome breakage. Such a comparison will reveal the desired information, namely, whether or not biological materials and physical instruments respond equally to the radiation. An example of such a presentation is shown in fig. 3; the data are from measurements made inside airplanes flown through atomic clouds at a number of different nuclear test detonations. The physical dose measurements by National Bureau of Standards calibrated film packs are being compared with the biological estimates of dose made at the same place in the same airplanes. Dr. Carter's estimates of dose, which are based on weight loss in mouse thymus glands (Carter *et al.*, 1954) are plotted along with the Tradescantia estimates, since the two materials were placed together, and it is of interest to see how the responses of different

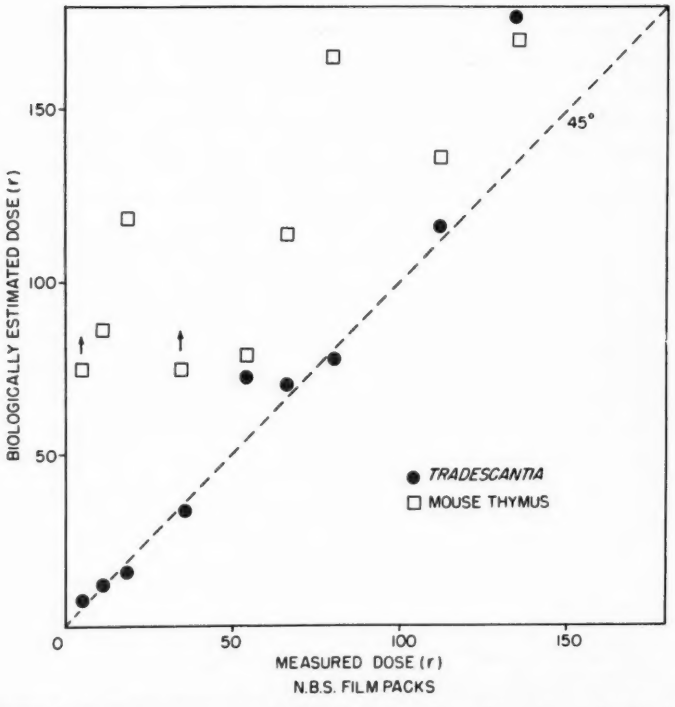

FIGURE 3. Dose measurements inside airplanes flown through atomic clouds. Physically measured dose (r) versus biologically estimated dose (r).

biological systems compare. On a plot of this type, of course, if there were a perfect correlation the experimental points would fall exactly on the 45° (dashed) line. The graph shows a number of things. In the first place, it can be seen that the mouse thymus estimates are considerably displaced at the lower doses. This is simply because their resolution is poor at these low doses, but it is better at the higher doses. And in fact, the Tradescantia and mouse thymus materials were an excellent complement to each other at the tests, for although Tradescantia was accurate to lower doses than the mouse thymus the mouse thymus was usable to higher doses than the Tradescantia, and there was a considerable region of overlap of the two systems.

The greatest single Tradescantia error is 24 per cent, at the highest dose, but at this particular dose (that is, in this particular airplane) both the mouse thymus and Tradescantia data agree, so this particular physical measurement may be in error. Other Tradescantia estimates agree with the physical data within 10 per cent or less, a remarkable degree of accuracy. Since the Tradescantia data as a whole, from lowest to highest doses, fall uniformly on, or almost on, the perfect correlation line, it can also be said that the radiation received inside an airplane from an atomic cloud has an RBE to gamma rays of about 1; for if the RBE were, for example, 2, the

points would fall along a line with an angle greater than 45° (actually about 63°). This does not occur. Furthermore, there is no systematic departure from the 45° line as dose increases, indicating that, over the range of dose involved, effect increases linearly with dose. It should be mentioned that the doses received in the individual airplanes were haphazardly distributed, and no particular correlation can be made between different airplanes.

We conclude from the airplane-atomic cloud results that (1) physical instruments will measure biological dose properly, (2) effect over the dose range observed increases linearly with dose, and (3) the RBE is about 1.

The second set of data, shown in fig. 4, is from exposure to nuclear test device gamma rays at increasing distances from ground zero. Here, again, there was good overlap of data from mouse thymus and Tradescantia, as well as dose measurements by two different physical instruments, films and ionization chambers, perhaps the best being the ionization chambers. For this set of data, unlike the airplane data just discussed, there is a definite relation between the doses received at different positions, which were at increasing distances from ground zero; so that we can compare not only the individual experimental points, but better still, the least-squares curves fitted to those points. The slope of these Tradescantia gamma-ray curves

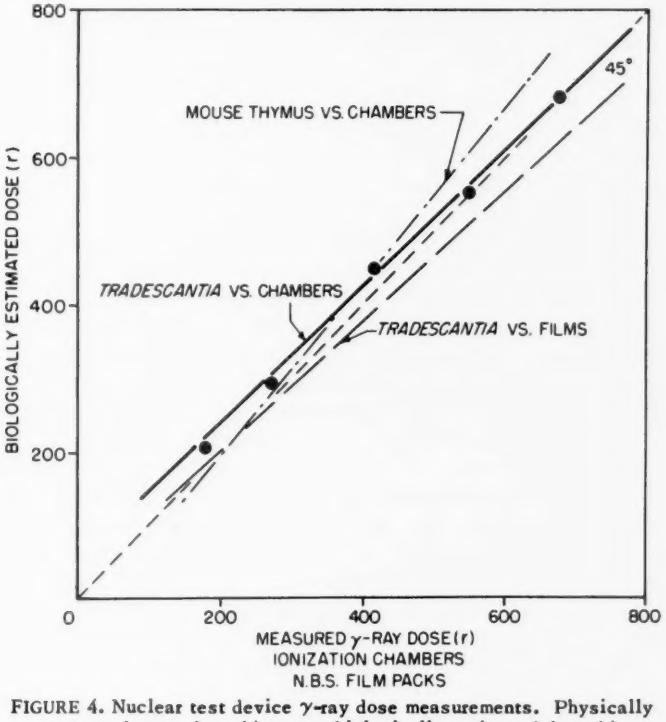

FIGURE 4. Nuclear test device γ-ray dose measurements. Physically measured γ-ray dose (r) versus biologically estimated dose (r).

against ionization chambers and against film packs have been compared and found not to differ significantly at the 5 per cent level. Tradescantia and Carter's mouse-thymus dose estimates are about 15 per cent apart in absolute units. Tradescantia is closer, in absolute units, to measurements by ionization chambers than to either the mouse thymus or film packs, but the greatest difference among any of the measurements is only 15 per cent over the rather considerable range analyzed biologically. For comparisons between biological and physical systems, between different biological systems, and for the range covered, these gamma-ray data are among the best that were obtained. The same conclusions can be drawn here as from the airplane data, namely, that the plant measured dose properly, that physical instruments can substitute for biological measurements of test gamma rays, and that the RBE of test gamma rays to hard X-rays is about 1.

The analysis of the fast neutron data is considerably more complex than the two cases just presented, and the principal difficulty at the first operation was a lack of sufficient physical data to compare with the living material. Fortunately, the gamma-ray and airplane results from the first operation were good enough so there was no need to repeat them, and at the second operation only neutron exposures were made.

The same exposure containers, with 7-inch-thick lead walls to exclude the gamma radiation but allow passage of the neutron radiation, were used for both operations. By various rather involved estimates and considerations it can be shown that *most* of the radiation received by the plants inside the containers was fast neutrons. Estimates of the amount of gamma-ray contamination received by the plants were poor for the first operation; they were better for the second, and show that about 25 per cent of the ionization was from gamma rays, but the true value is probably less at the outer stations where Tradescantia was exposed.

For these neutron stations, there were measurements of the amount of effect caused in Tradescantia and of the "dose" (actually, the number) of neutrons. The amount of damage caused by test neutrons, when compared with the appropriate control data (from the laboratory experiments with measured doses of neutrons or X-rays), can be expressed as: (1) the estimated dose of *neutrons* (in n units) that would cause equal effect, or (2) the estimated dose of *X-rays* (in roentgens) that would cause equal effect.

The first neutron graph, fig. 5, shows the relation of biologically estimated dose against neutron "dose." The treatment is the same as that used previously, except that biological and physical measurements are in different, though proportional, units. Since the test neutron dose is expressed in relative units, the only test of the accuracy of the biological dose estimates is how well the points fit a straight line. The line "Tradescantia n vs. flux" is the estimate of that dose of *neutrons* which would produce the same amount of effect as the test neutrons. This first line has a reasonably good fit and shows therefore that Tradescantia is properly integrating the neutron dose over the range tested. But as was just mentioned, an estimate can also be made of the dose (in roentgens) of X or gamma rays

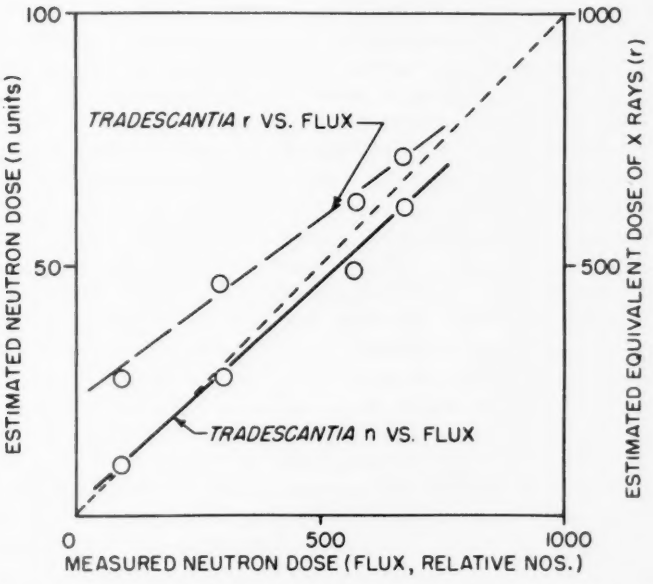

FIGURE 5. Nuclear test fast neutron dose measurements. Physically measured neutron "dose" (flux, relative numbers of neutrons) versus biologically estimated neutron dose (n units) and estimated equivalent dose of X-rays (r).

that cause equal effect; this is shown in the second curve of "Tradescantia r vs. flux." It should be noted that these two lines converge toward the higher doses, which should be so since, as was stated earlier, these chromosomal aberrations increase linearly with dose of neutrons but quadratically with X or gamma rays. This last curve was useful for intercomparisons with other biological material exposed along with Tradescantia. The Tradescantia results were in agreement with other physical measurements and parameters, too, such as decrease of neutron flux with distance, and were useful for prediction of magnitude of biological effect as a function of distance. But the lack of physical measurement of tissue dose makes it impossible to compare the test neutron results with the control data and derive relative biological efficiency, as was possible with the airplane and gamma-ray data.

At the second nuclear test operation, biological and physical measurements can be expressed in the same units, namely, rep, or tissue dose. This is possible because C. W. Sheppard and E. B. Darden (see Kirby-Smith and Swanson, 1954) developed a neutron ionization chamber calibrated in rep which was used for both the control experiments (with cyclotron neutrons) and the test exposures. The Tradescantia work for this second operation was done by J. S. Kirby-Smith and C. P. Swanson (1954). The fast neutron ionization chambers were calibrated at Oak Ridge and were shown to measure neutron ionization properly and to be essentially energy independent

(less than 10 per cent) over the range of energy experienced. Tradescantia and the Sheppard-Darden neutron ionization chambers were exposed together to test neutrons inside the lead containers, which gave, along with the control data, the following measurements: (1) ionization chamber dose in rep for control and test exposures and, (2) Tradescantia effect, chromosomal aberrations, for control and test exposures. With these data it is possible to compare physical measurements versus the biological estimates of test neutron dose.

Such a comparison from Kirby-Smith's and Swanson's data is shown in fig. 6, the same biological-physical correlation made previously. The different symbols are estimates from the different types of chromosomal aberrations. It is seen that, over this 50-fold range of dose, Tradescantia is measuring dose properly within about 10–15 per cent of the physical instruments. To express this range in more familiar terms, the effect observed at the least and greatest neutron doses corresponds to what would be caused by X-ray doses of 18 and 780 r. A line fitted to the test data (the solid line) falls somewhat above the 45° (that is, 1:1) line, but 25 per cent is the greatest possible difference between these test and control neutron results, meaning that test neutrons could be at the most only 25 per cent more effective biologically than the cyclotron neutrons.

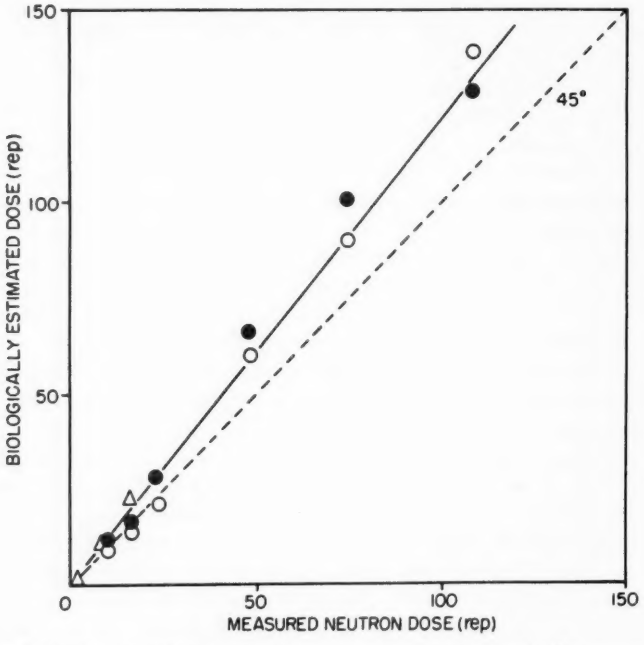

FIGURE 6. Nuclear test fast neutron dose measurements. Physically measured neutron dose (rep) versus biologically estimated dose (rep).

Some of the possible errors that might be involved in these neutron experiments should be mentioned, if only to show that they have been considered and are not pertinent to these values and conclusions. These comments are meant to apply only to the Tradescantia range of doses at the most distant stations; the situation becomes more complex at closer distances, that is, at greater doses. If there were any great difference in neutron energy spectrum between the test and control experiments, and either the chambers or Tradescantia were energy dependent, the values could be in error. But physical measurements have shown that the two spectra are effectively about the same, and anyway the chambers are energy independent, or practically so, over the range encountered. The neutron energy dependence of Tradescantia is not known quantitatively. Secondly, if there were an appreciable amount of gamma-ray contamination mixed with the fast neutrons, the results could be seriously in error, since the chambers respond equally to neutrons and gamma rays, but Tradescantia is affected only a tenth as much by gamma rays as by neutrons. The physical estimates are that about 10–25 per cent of the ionization in the Tradescantia range was gamma rays. Also, it can be shown biologically that the gamma-ray contamination must be about this or less, by the following argument. Because the chambers measure neutrons and gamma rays equally, it can be seen that the same total ionization dose measurements would be obtained whether the radiation were 100 per cent neutrons, or 80 per cent neutrons plus 20 per cent gamma rays, or 50:50, and so on. But the Tradescantia response to equal total doses (as measured by the chambers) would become less and less as the amount of gamma-ray contamination became larger and larger. If, say, the radiation were half neutrons and half gamma rays, the Tradescantia effect would be reduced to only 55 per cent of the effect from an equal ionization dose of pure neutrons. This is so because, although the half dose of neutrons would cause exactly half as much effect, the other half dose of gamma rays would cause only a half divided by 10 (the RBE of neutrons to gamma rays), or 5 per cent as much effect, making the total only 55 per cent the effect if the dose were pure neutrons. So in fig. 6 a gamma-ray contamination of 10 per cent would make the curve fall to 91 per cent of the pure neutron yield, 20 per cent to only 82 per cent, and so on. And it is apparent from the graph that, if anything, the exact opposite is true and the test neutron curve is higher.

Statements cannot be made about the physical situation in the detonation test neutron studies with the same certainty as for well-controlled laboratory experiments; the conditions are such as to make a physicist, who is accustomed to 1 per cent accuracy and certain knowledge about the variables involved, quite unhappy. Nor is it the happiest situation for a biologist, who can under good conditions achieve 5 per cent accuracy. But for the purpose of the nuclear test device biological experiments, a 10–15 per cent error is a small one, and amounts to very satisfactory results. Striving for greater accuracy would be unnecessary and wasted effort.

One last test of the Tradescantia experimental methods is worth mentioning. Since the dose estimates are based on a comparison of the amount of chromosomal breakage caused in another part of the world by a nuclear test device with the amount caused in the control experiments done in the laboratory at a different time, it is important to establish that the plant had not altered its response to radiation under the field conditions. To a physicist, this amounts simply to an instrumental calibration. So a specific control experiment was made under field conditions in exact simulation of a nuclear test, except that a measured dose of radiation was given to the plants. And it was found that a dose of radiation given under the specific field conditions of the nuclear detonation tests produced about the same effect in Tradescantia as did an equal dose in Oak Ridge; for example, the Tradescantia estimate of a measured dose of 100 r of hard X-rays under test conditions was 99 r from one type of aberration and 107 r from another type. It is gratifying that in a sample experiment of this sort (which is really a test of the methods of analysis being used for the nuclear detonation data) there is such a good agreement between estimated dose and the known actual dose.

It would appear that it can now be said, in answer to the questions asked at the beginning, that nuclear test detonation radiation has not produced any new or novel effects; that Tradescantia has shown, as far as the gamma-ray and airplane experiments are concerned, that physical instruments will serve as accurate measurers of biological dose; and that neither test gamma rays nor neutrons, at least over the considerable biological range of doses studied, have an RBE which is significantly different from that known from laboratory sources of these radiations. Under rather difficult conditions, Tradescantia has functioned as a dosimeter with an average accuracy of 10–15 per cent.

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THE THEORY AND APPLICATION OF A NEW METHOD OF DETECTING CHROMOSOMAL REARRANGEMENTS IN *DROSOPHILA MELANOGASTER*[1]

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A new method of detecting chromosomal rearrangements in *Drosophila melanogaster* has been applied to the problem of measuring the biological effects of ionizing radiations from nuclear detonations. The method, itself, is an outgrowth of studies of the bithorax pseudoallelic genes near the middle of the right arm of the third chromosome (Lewis, 1951). It will be called the "bithorax" method. Results of applying it to the detection of X-ray induced rearrangements will be considered first.

MATERIALS AND METHODS

The bithorax method employs two mutant genes of the bithorax pseudoallelic series; namely, the recessive, bithorax-34e (bx^{34e}, locus 58.8) found by Schultz (Bridges-Brehme, 1944) and the dominant, Ultrabithorax (*Ubx*, locus 0.02 unit to the right of *bx*) found by Hollander (loc. cit.) who named it bithorax-Dominant (bx^{D}). The name Ultrabithorax is intended to supersede the original name and also the name Bithorax-like (*Bxl*) by which it has also been known (Lewis, 1951).

The bx^{34e} homozygote has a slight but highly constant and symmetrical mesothoracic-like modification of the metathoracic segment. The dorsal metathorax or "metanotum" which in wild-type flies is little more than a "line" separating the mesothorax from the abdomen (see Zalokar, 1947), is transformed in the bx^{34e} mutant flies into a narrow band of hairy and bristled tissue (fig. 1). The halteres in the mutant flies are somewhat enlarged and slightly wing-like. The *Ubx* gene is lethal when homozygous while the heterozygote, *Ubx*/+, differs from wild-type chiefly in having the distal segment of the haltere enlarged to about twice its normal volume. Since bx^{34e} and *Ubx* are pseudoallelic genes there are two forms of the double heterozygote to be considered; namely, the cis- and trans- forms—a useful terminology proposed for such cases by Pontecorvo (1950). The cis- form, or bx^{34e} *Ubx*/+ +, cannot be distinguished phenotypically from the + *Ubx*/+ + heterozygote just described. On the other hand the trans- form or bx^{34e} +/ + *Ubx* has oval, flat halteres which are consistently larger and much more wing-like than those of the bx^{34e} homozygote. Paradoxically, however, the trans-heterozygote (or "trans-type" as it will be referred to hereafter) has

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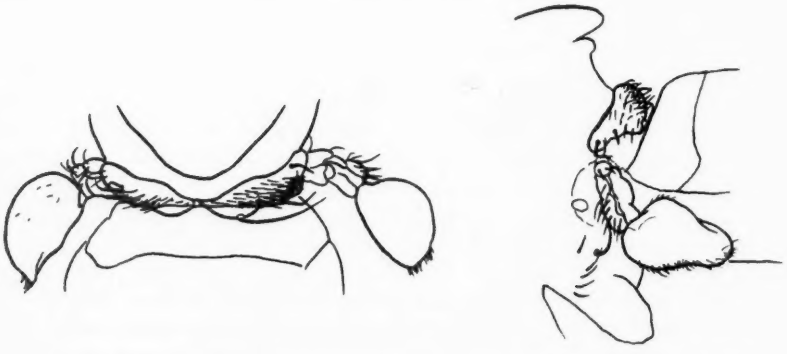

FIGURE 1. Dorsal (left) and lateral (right) views of the metathoracic region of the bx^{34e} homozygote. (Drawing by E. M. Wallace.)

virtually no metanotal tissue such as is seen in the bx^{34e} homozygote. In other words, the trans-type is at once more extreme and less extreme in the degree of its bithorax effects than the bx^{34e} homozygote, depending upon whether the haltere or the metanotum is considered.

As will appear, the extent of development of the metanotum in the trans-type is extraordinarily sensitive to certain structural rearrangements of the third chromosome. The following rough, arbitrary system has been developed for measuring this phenotype: grade 0 indicates no appreciable development of the metanotum; grade 1 indicates tufts of hairy metanotal tissue which do not, however, form a continuous band across the dorsal side of the thorax; grades 2, 3 and 4 indicate progressively wider, continuous bands of such tissue—grade 3 having approximately the width seen in the bx^{34e} homozygote (fig. 1) and grade 4 having a width about that of the first abdominal segment, to use a convenient reference point. The procedure is to score the grade of the trans-types which arise from a mating of bx^{34e} homozygotes with *Ubx*/+ heterozygotes (the chromosome carrying the normal alleles of these genes should also be marked and in the experiments reported below carried the "balancer" Payne, *Dfd;* that is, carried the dominant Deformed (eye) mutant and the Payne inversions in the left and right arms of the third chromosome). Either of these genotypes may be used as the treated or male parent but the bx^{34e} homozygote is the one more usually used. In the fast neutron, gamma-ray, and nuclear detonation studies, bx^{34e} males were treated and mated to *Ubx*/+females, which carried an attached-X type of X chromosome—the so-called double-X of Muller; in these cases only the F_1 male trans-types were scored. Standard culture bottles were used; they were incubated throughout at 25°C, and crowding was avoided (for example, the controls had only one pair of parents). These environmental conditions are essential for maximum sensitivity of the method.

In order to minimize subjective errors in grading the trans-types, the control and treated culture bottles were given randomly drawn numbers by another person who also removed all of the parental flies before emergence of

the F_1 generation. This coding procedure was followed in experiments designed to calibrate the method for different types of radiations. Finally, the scoring and grading of all flies was done throughout by one person (the author).

Cytological analyses were conducted by examining the salivary gland chromosomes of individuals heterozygous for the treated chromosome (containing either bx^{34e} or *Ubx*, as the case might be) and an untreated chromosome of normal sequence carrying a mutant at another of the bithorax pseudoallelic loci, namely, bithoraxoid (*bxd*). The latter mutant permitted the bx^{34e} and *Ubx* containing third chromosomes to be readily distinguished from one another in the larval stage; thus, bx^{34e} +/+ *Ubx* males are outcrossed to bithoraxoid females to produce bx^{34e} +/+ *bxd* larvae which are wild-type in phenotype, and *Ubx* +/+ *bxd* larvae which lack setae on the first abdominal segment. Salivary gland chromosome map designations were made from Bridges' 1935 map.

The source of X-rays was a Westinghouse 150-KV Industrial Unit employing a Machlett radiographic tube which was operated in all cases at 120 KV, 8 ma., with a 1 mm Al filter. Exposure of flies to gamma rays (from Cobalt-60) and to fast (pile) neutrons were carried out at the Argonne National Laboratory. For the X-ray studies the males were put into small gelatin capsules; while for all of the other types of treatments males were transported (by air) and treated in cotton-plugged polystyrene plastic tubes ($2\frac{1}{2}$" long; $\frac{1}{2}$" diameter; and $\frac{1}{16}$" wall thickness). In all of these latter cases, the males were received at the laboratory and mated within 24 hours after the treatments. Such males were removed and discarded on the seventh day after treatment.

X-RAY EXPERIMENTS

The results of grading the metanotum of trans-types in the F_1 generation following treatment of adult parental males are shown in table 1. It is apparent that a significant number of the trans-types from each X-ray treatment fall into grades 2–4, inclusive; while none of the controls fall into these grades. In this latter connection it is noteworthy that all of the control and 3,000 r cultures in these experiments were pooled and coded by the method already described. Among 278 individuals falling into grades 1–4, inclusive, in these experiments, the ratio of females to males was 124:154. It is probable that the excess of males is an expression of a slight sexual dimorphism in the phenotype.

The vast majority of trans-types which belong to grades 2–4, inclusive, transmit an average grade significantly greater than the approximately grade-0 average of the control trans-types. On the other hand many trans-types belonging to grade 1 fail to show inheritance of the effect upon appropriate testing; *e.g.*, in the 3,000 r experiment, 19 grade-1 males were individually progeny tested by mating to bx^{34e} females; of these tests, 13 were fertile and only six transmitted the effect (usually averaging about grade 1) to the F_1 trans-types. In general, the average grade of the trans-types produced in

TABLE 1

THE GRADING OF TRANS-HETEROZYGOTES

Treatment	Dosage	Phenotypic grades						Per cent grades 2–4, inclusive	Standard deviation (per cent)
		0	1	2	3	4	Total*		
	Control	2,559	4	0	0	0	2,563	0.00	....
X-rays	3,000 r†	2,917	33	45	16	9	3,020	2.32	± 0.26
(120KV)	4,500 r	2,667	48	97	25	6	2,845	4.50	± 0.39
Gamma rays	Control	1,303	9	0	0	0	1,312	0.00	....
(from Co^{60})	3,000 r	2,274	28	30	5	4	2,341	1.67	± 0.26
Fast	200 rep	1,823	35	20	4	0	1,882	1.28	± 0.26
neutrons	400 rep	2,117	40	24	5	1	2,187	1.37	± 0.25
(from Ar-	800 rep	1,325	44	37	13	3	1,422	3.73	± 0.50
gonne pile)	1600 rep	299	37	19	8	4	367	8.45	± 1.45
	Control	398	0	0	0	0	398	0.00	....
Nuclear	(70 rep)	869	5	3	0	0	877	0.34	....
detonation	(150 rep)	1,159	11	8	1	0	1,179	0.76	± 0.25
(mostly	(190 rep)	1,114	26	8	3	0	1,151	0.96	± 0.29
fast	(330 rep)	1,202	23	18	2	1	1,246	1.69	± 0.36
neutrons)	(940 rep)	646	29	28	6	0	709	4.80	± 0.80
	(1100 rep)	177	26	8	3	1	215	5.58	± 1.57

*In the X-ray series and its control, males and females are included in the totals. In all of the remaining experiments the totals refer only to males.

†In this experiment the treated male parent was *Ubx*/+; in all other cases the male parent was homozygous for bx^{34e}.

the F_1 of a progeny test is similar to, or within one grade unit of, that of the parental trans-type. A small percentage of asymmetrical individuals arise. These are usually cases in which one-half of the body is of grade 0. Such cases are scored as grade 0 and have rarely transmitted the higher grade. The remaining asymmetrical types are classified according to the lower grade present since that grade is the one usually transmitted in such cases. In general, a population of a given trans-type shows little tendency towards asymmetrical metanotal development.

The next step was to determine what proportion of the trans-types belonging to the different graded phenotypes carried chromosomal rearrangements. A total of 128 treated third chromosomes (obtained from progeny-tested male trans-types) were analyzed by the salivary gland chromosome

TABLE 2

CORRELATION OF GRADE OF MALE TRANS-HETEROZYGOTE WITH X-RAY INDUCED REARRANGEMENT (R) INVOLVING THE THIRD CHROMOSOME

Original grade of male	R absent	Critical region	R involving non-critical region	Total
0	33	0	4	37
1	5	9	0	14
2–4	3	74	0	77
				128

method. (This total includes 74 cases from preliminary 4,000 r X-ray experiments, not shown in table 1, in which the cultures were scored by another person.) The results are shown in table 2. Among 37 trans-types belonging to grade 0, only four (10 per cent) carried rearrangements involving the treated third chromosome. On the other hand, among 91 trans-types belonging to grades 1–4, inclusive, 83 (91 per cent) carried rearrangements involving that chromosome. Moreover, a striking difference in the distribution of breakages in the third chromosome is evident. Thus, every rearrangement associated with a trans-type of transmitted grade of 1 or higher had at least one breakage point in the region extending from the centromere to immediately to the left of the section including the *bx* and *Ubx* loci (89E; Lewis, 1951); that is, in the region from section 81F to 89D, inclusive. This relation will be referred to here as Rule (1). The region will be called the "critical region," and comprises 506 discs or bands as measured on the revised map of Bridges (1941). On the other hand, Rule (2), in each of four cases in which the rearrangement was associated with a grade of 0 the breakage point in the third chromosome fell outside of the critical region (the sections being, 64F, 76D or E, 80, and 94D). The eight instances of transmission of the altered grade which were not associated with visible rearrangement require comment. Five of these cases were of grade 1 to begin with and were transmitted as grade-1 types. These may have been due to changes in modifier genes or to rearrangements not readily detectable by the salivary gland chromosome method (such as, wholly heterochromatic exchanges). The three cases of this kind among the higher grades were all of grade 2 and were transmitted as grade-2 types. One proved to be caused by, or associated with, a second chromosome Minute (bristle) change (but four other known Minute types which were tested did not act as modifiers *per se* of the trans-type). The other two cases without rearrangements have not been analyzed further but one has been preserved in stock.

Two-, three-, four-, and five-break rearrangements occurred in the ratio of 53 : 14 : 13 : 3, respectively, among the 83 rearrangements discussed above (table 2). The multiple-break, as well as the two-break, cases were distributed throughout all grades but the former were proportionately more often associated with the higher grades of 3 and 4 than were the latter.

A number of features stand out clearly when a plot is made of the distribution of breakages among the two-break rearrangements detected by the bithorax method. A total of 81 such cases were available from the X-ray experiments. (This total includes 53 cases from table 2 and 28 cases from progeny tests of female trans-types.) The result is shown in table 3.

Two more general rules may now be formulated from the pattern of breakages seen in this table. Thus, Rule (3), when one breakage point occurs in the proximal half of the critical region (from section 81 to 85 or 86, inclusive), the other breakage point tends to occur in the distal half of one of the other major euchromatic chromosome arms (X, 2L, 2R, or 3L). On the other hand, Rule (4), when one breakage occurs in the remaining or distal part of the critical region (from 86 or 87 to 89D, inclusive), the other break-

TABLE 3

POSITION OF BREAKAGE IN X-RAY INDUCED TWO-BREAK REARRANGEMENTS DETECTED BY THE BITHORAX METHOD. EACH ENTRY IS THE NUMBER OF THE SALIVARY GLAND CHROMOSOME BREAKAGE SECTION IN THE NON-CRITICAL REGION; WHEREVER TWO OR MORE VIRTUALLY IDENTICAL REARRANGEMENTS OCCUR, THE NUMBER OF SUCH CASES IS SHOWN IN PARENTHESIS.

Distance from tip of second break*	Number of the breakage section in critical region									Total (by arm)						
	81F	82	83	84	85	86	87	88	89	X	Y	2L	2R	3L	3R	4
1	60(2)		60	61		60	21; 61					1	4	2		
2	22(3)											3				
3	3; 58(2); 63(3)	63	3					63	98	2			2	5	1	
4	57(2); 64(2)	64											2	3		
5	5; 25; 56(3); 65									1		1	3	1		
6	55(2)												2			
7	67(2)		60										1	2		
8	68	28; 53				8		8		2		1	1	1		
9	52												1			
10	30(2); 70(2)			70±†	30			30				4		3		
11	91							11		1					1	
12							62							1		
13				48		33						1	1			
14																
15					75	75		35				1		2		
16																
17						37±		37±				2				
18																
19						39±	39					2				
20						Y‡; 80±	20; Y; 41; 101	Y; 40(2); 41 80±(2); 101	Y(2); 41(3) 80; 80±	1	5	2	5	5		2
Total	32	4	3	3	2	8	8	13	8	7	5	18	22	27		2

*These distances are in terms of the number of salivary gland chromosome sections from the tip for any given major chromosomal arm.

†The sign ± indicates that the breakage location was only roughly made to the nearest section; 80± signifies that the breakage is in either section 80 of 3L or, less likely, in 81 of 3R.

‡Y symbolizes a breakage position in the heterochromatic Y chromosome, which corresponds roughly to section 20 of the X chromosome.

age point may occur apparently anywhere in the entire chromosome complement (including Y, 4 and 3R). The phenotypic grades seem to be correlated roughly as follows: for the same breakage position in the critical region, the more distal the break in the other arms the more extreme the grades; while for the same breakage position relative to the centromere in other arms, roughly speaking, the more distal the break in the critical region the more extreme the grade (although there is no definite trend in the proximal one-half of the critical region in this respect).

The bithorax method is unique among the known position effect methods in that it detects wholly euchromatic rearrangements with considerable efficiency. Thus, from table 3, among the 81 two-break rearrangements 29 (36 per cent) were of this type (not involving breakages in the heterochromatic sections: 20, 40, 41, 80, 81 nor 101). In spite of this result, the method is seen to be very inefficient at detecting inversions in the right arm of the third chromosome. Thus, only two of these were detected; yet from the data of Bauer (1939) it is known that significantly more inversions involving 3R occur than translocations between 3R and any one of the other autosomal arms when rearrangements are detected by the salivary gland chromosome method.

Finally, aside from the broad relationship laid down by Rule (3) above, there is no significant evidence that breaks in the critical region tend to be preferentially associated with specific regions of other chromosomes, when allowance is made for the fact, well established by Bauer (loc. cit.), and Kaufmann (1946), that certain regions normally show a relatively higher breakage frequency than their salivary gland chromosome physical distance would indicate (such as section 3 in X and any heterochromatic section such as 41).

The validity of the above defined rules can best be tested by employing unselected rearrangements; *i.e.*, those selected by other methods of detection. For this purpose the available two-break rearrangements were surveyed and the required R (bx^{34e} +) or R (+ *Ubx*) chromosome rearrangement (R) types were derived as crossover products from R (+ +)/bx^{34e} + and R (+ +)/Ubx +, females, respectively. The breakage points in such rearrangements were either already known (Bridges-Brehme, loc. cit.) or in some cases given below were determined for this study.

Three translocations (T), not involving the critical region, were used to test the validity of Rule (2); namely, T(1;3) *v* (10/93B), (the latter set of numbers in parenthesis indicating the breakage points in terms of salivary gland chromosome regions), T(2;3)S^{M} (21E/79D), and T(2;3) $bw^{VD\bullet4}$ (59D/80). None of these rearrangements enhanced the bithorax phenotype of the trans-type; that is, R (bx^{34e} +)/+ *Ubx* for each of the three rearrangements is phenotypically like the trans-types which carry no rearrangements involving the third chromosome. Secondly, every rearrangement tested whose breakage points agree in type with those described by Rule (1) enhanced the grade of the trans-type; of these, the examples obeying Rule (3) were: T(2;3)$bw^{VD\bullet3}$ (59E/81F), *T(2;3)Dp-S* (21E/81F; Lewis, 1945); and

the inversion, In (3LR) *sep* of Muller (65E/85E); while an example obeying rule (3) or (4) was T(3;4)c (86B-C/101F). Finally, two unselected rearrangements having breaks in the proximal part of the critical region but the other breaks not in conformity with Rule (3) are of interest; namely, T(2;3)A (39B or C/83B), and T(2;3)B (33/81F). The former is barely detectable as a modifying rearrangement of the trans-type, while the latter is just detectable by its modification of the trans-type to an average grade of about 1.

Preliminary (4,000 r) X-ray experiments had shown that the frequency of phenotypically modified trans-types was roughly the same whether the treated male parent was bx^{34e} or *Ubx*/+. This indirect evidence suggested that R (bx^{34e} +)/+ *Ubx* was equivalent to R (+ *Ubx*)/bx^{34e} + for the identical type of R. This rule has been verified repeatedly in this work using both the selected and unselected rearrangements available. The necessary R (bx^{34e} +) and R (+ *Ubx*) crossovers were, for example, both obtained in the case of T(2;3)$bw^{VD\circ 3}$ and T(3;4)c. In the case of the former rearrangement the two kinds of trans-types are mostly in the range of grade 3 and 4 and are not phenotypically distinguishable from one another. In the latter case of T(3;4)c, the two trans-types are again indistinguishable from one another phenotypically and range from grade 0 to grade 2.

Thus far, only heterozygosity for structural changes has been considered. A number of rearrangements selected by the bithorax method have proved viable when homozygous so that it becomes possible to compare structural homozygotes of the general genotype, R (bx^{34e} +)/R (+ *Ubx*), with the structurally heterozygous trans-types. More often, however, it has been necessary to cross two nearly identical rearrangements, one associated with bx^{34e} and the other with *Ubx* in order to approach structural homozygosity for the rearrangement sequence. The results of such studies have indicated, almost without exception, that structural homozygosity restores the grade of the trans-type to its original grade-0 condition—Rule (5). Thus, the selected rearrangements, T(2;3) 39–86, bx^{34e} (39/86C-D) and T(2;3) 39–87, *Ubx* (39A-C/87A-C) produce when combined a trans-type of the original grade, although each by itself gives a grade average of about 2. Exceptions to Rule (5) are very instructive and are illustrated by the following two examples: In (3LR)64-82 (64C/82A) and T(2;3) 57–81 (57D-E/81F). Each is viable when homozygous and each has been used to make a structurally homozygous trans-type, which proves to be predominantly of grade 1 or 2 in its phenotype instead of grade 0. All of such exceptions thus far have been cases in which the bithorax region has been shifted to a much more distal location than it normally occupies. Thus, in the case of the homozygote for the pericentric inversion and the homozygote for the translocation which have just been discussed, the bithorax region (89E) now is located more than 1220 discs and more than 1450 discs, respectively, from the centromere compared to its normal location of 506 bands.

At the other extreme, when the intensity of structural heterozygosity in the trans-type is increased by combining two quite different kinds of two-break rearrangements, the phenotype tends to become very extreme. For

example, the translocation, T(2;3) 48–84, bx^{34e} (48C/84D) has been combined with T(3;4)c, *Ubx* and with *T(2;3)* 29–87, *Ubx*, already described. In each case the phenotypic grades of the double structural heterozygote are in the range of 3 and 4, whereas the three heterozygotes by themselves each have grades of mostly 1 and 2.

One rare type of rearrangement must be considered in applying the bithorax method. Rearrangements associated with breakages immediately adjoining the 89E1-2 doublet act like very extreme *bx* and *Ubx* mutations or position effects. A number have been obtained from X-raying wild-type males, or bx^{34e} males in other kinds of experiments. When bx^{34e} males are treated and mated to the *Ubx*/+ female, as in the bithorax method, such changes are expected to occur but to be lethal when opposite the Ubx chromosome from the female. On the other hand when the reciprocal cross is made a trans-type carrying a rearrangement of this kind is expected to result on rare occasion. Two of such cases were detected in the 3,000 r experiment shown in table 2 as very extreme grade-4 individuals. The effect of such cases on the per cent of individuals falling into grades 2–4, inclusive, is however negligible.

The addition or subtraction of a Y chromosome does not noticeably alter the grade of the trans-type either in the presence or absence of structural heterozygosity for rearrangements involving the critical region. An example was the translocation, T(2;3)bw^{VDe3} which was studied in the XXY and XO male in the form, R (bx^{34e} +)/+ *Ubx*, in each case the phenotype remains of grade 3 or 4, even though the variegation for the normal allele of the brown gene (bw^{+}) in this case shows the expected modification due to changes in numbers of Y chromosome.

An important feature of the bithorax method is the fact that its rearrangements only modify the phenotype of the trans-type. Thus, e.g., R (+ +)/ bx^{34e} +; R (+ +)/+ *Ubx*; R(bx^{34e} +)/bx^{34e} +; and R (+ +)/bx^{34e} *Ubx* have in each case proved phenotypically indistinguishable from the corresponding genotype without the rearrangement. This feature holds in particular for such rearrangements as T(2;3)bw^{VDe3}, which, as already noted, is an extreme modifier of the grade of the trans-type.

FAST NEUTRON AND GAMMA-RAY EXPERIMENT

It was anticipated that the flies to be exposed to the nuclear detonation would receive chiefly fast neutron radiation; *i.e.*, they would be shielded by inclosure in lead chambers. For this purpose it was obvious that the bithorax method of detecting rearrangements had to be calibrated in terms of known dosages of fast neutrons. The results of exposures of adult males (bx^{34e} homozygotes) to a series of known dosages of Argonne pile neutrons and to a 3,000 r dosage of gamma rays (from a Co^{60} source) are included in table 1. All of the fast neutron, gamma-ray and control cultures in this experiment were pooled and coded by the method already described. Only the F_1 male trans-type were scored.

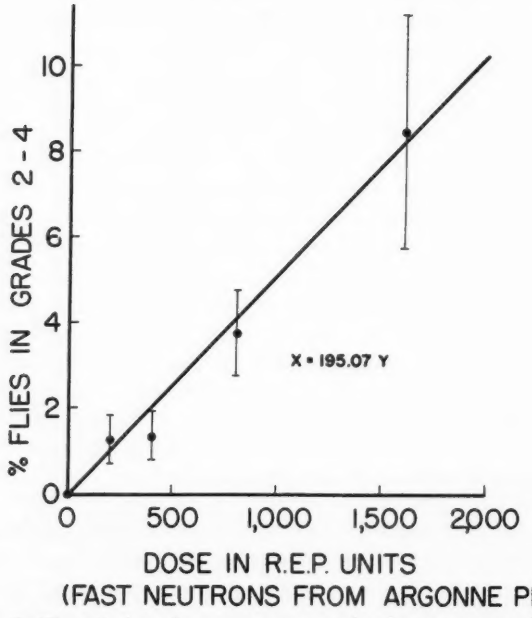

FIGURE 2. The relation of rearrangement production as measured by the percentage flies in grades 2–4, inclusive, with dosage of fast neutrons in roentgen-equivalent-physical (r.e.p.) units. For each experimental point the 95 per cent confidence interval is shown.

The fast neutron results show a reasonable fit to a linear relationship with dosage in rep (roentgen-equivalent-physical) units (fig. 2). The data have been fitted by the method of least squares to a linear curve of the form $x = ay$ (that is, to a curve which, on *a priori* grounds is expected to pass through the origin) where x is the dosage in rep units and y is the per cent of individuals falling into grades 2–4, inclusive. The value of the constant, a, fitted in this way is 195.07. This curve served as the best available prediction curve for the unknown dosages received at the nuclear detonation sites.

NUCLEAR DETONATION EXPERIMENT

The results of applying the bithorax method to the detection of rearrangements produced by the fast neutrons (contaminated to some extent by gamma rays) resulting from one of the nuclear detonations are also included in table 1.

The dosage that the flies received at various stations has been estimated simply by substituting the values of the percentage of flies falling into grades 2–4, inclusive, into the equation, $x = 195.07\ y$, which was the curve obtained above when the bithorax method was calibrated with known dosages of fast neutrons. The values of x thus obtained (and rounded to two

significant figures) are shown in parenthesis in the column headed "Dosage" of table 1. The validity of this procedure rests of course on a number of assumptions not too easily tested; namely, (1) that fast neutrons from the nuclear detonation will be identical with those from the pile in their rearrangement inducing capacities for Drosophila, and (2) that the percentage of gamma-ray contamination in the two cases is also the same. Nevertheless, these biological estimates derived from the bithorax method of the dosages received at the various test stations are found to agree quite well in each case with physical measurements.

DISCUSSION

The new type of position effect which underlies the bithorax method of detecting chromosomal rearrangements has important implications for the theory of the position effect phenomenon (review by Lewis, 1950). Some of these implications will be briefly discussed here.

Essentially, the new type of position effect is closely related to that revealed when cis- and trans-heterozygotes for certain pairs of pseudoallelic genes are compared. Thus, in the latter type of position effect (Green and Green, 1949; Lewis, 1945; 1951; 1952) the normal alleles of two such genes function better when both are together in the same chromosome. A model for this type has already been discussed in some detail (Lewis, 1950; 1951; see also similar speculations by Pontecorvo, 1950). It postulates that (1) a product A produced by gene, a^+, is utilized in some way by gene, b^+, to make a product B; and (2) A is effectively transported only along the chromosome (see fig. 3). This type of position effect will be referred to here as the "cis-vection effect."

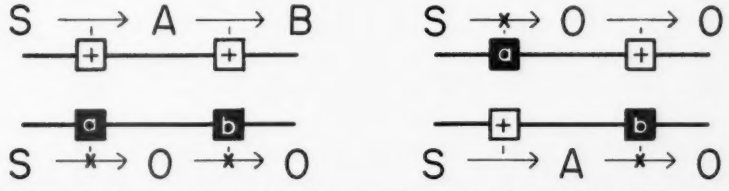

FIGURE 3. Diagram illustrating the possible mechanism of the position effect associated with the cis- (left) and trans-heterozygote (right) for certain pairs of pseudoallelic genes. For simplicity, the mutant genes, a and b, shown as black squares, are assumed to result in complete blockages of the reactions under their control, and the substance A is assumed to show no diffusion from one homologous chromosome to the other. The normal alleles of the mutant genes are signified by squares containing "+."

On the above model the intensity of the cis-vection effect, measured by the degree of phenotypic difference between the cis- and trans-heterozygotes, might be expected to be a function of the distance separating homologous chromosomes. Specifically, in an organism with somatic pairing, such as Drosophila (Metz, 1916), there may be a greater possibility of transport of A from one homologue to the other than in an organism without somatic

pairing. This consideration led to the experiment of testing whether rearrangements which might upset the pairing of the chromosomes in the bithorax region would alter the phenotype of the trans-heterozygote. This prediction has been fully verified by the above rearrangement studies employing the bx^{34e} and *Ubx* genes. The position effect that is revealed by modifying the trans-heterozygote by means of chromosomal rearrangements will be referred to as the "trans-vection effect." A diagram of a model for this effect is shown in fig. 4.

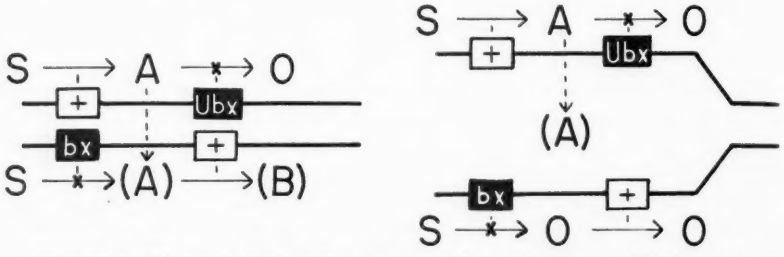

FIGURE 4. Diagrams illustrating the possible mechanism underlying the new type of position effect seen in the bithorax method of detecting rearrangements. The diagram on the left shows the postulated significance of somatic pairing in promoting the transport of substance A from one homologue to the other of a trans-heterozygote between bithorax (*bx*) and Ultrabithorax (*Ubx*) mutant genes. In the diagram on the right a reduction in somatic pairing, such as might be caused by a heterozygous rearrangement proximal to these genes, is assumed to prevent the "leakage" of A from one chromosome to the other.

The principal rules governing the trans-vection effect have been seen to hold for both unselected and selected rearrangements and have reasonable interpretations on a somatic pairing basis. Thus, Rules (1) and (2), that a rearrangement to be effective in significantly modifying the phenotype of the trans-type must have one breakage point in the critical region, suggest that somatic pairing is initiated at the centromere, or in the chromocentral regions, and then proceeds distally. Rule (3), that a breakage in the proximal part of the critical region tends to occur with another breakage in the distal half of an autosomal arm, suggests that owing to the formation of a chromocenter, or proximal association of non-homologous arms, rearrangements with both breakages in the proximal regions of different arms give relatively little failure of pairing (just as wholly heterochromatic translocations are virtually undetectable in the salivary gland chromosomes). Rule (4), that a breakage in the distal part of the critical region may occur with a breakage apparently anywhere else in the complement, suggests merely that the remaining distance between the position of the former breakage and that of *bx* and *Ubx* genes is so short that the probability of somatic pairing occurring in this interval is made relatively small. Finally, Rule (5) that structural homozygosity does not in general modify the phenotype of the trans-type is obviously expected on a somatic pairing basis. Exceptions to this later rule involved rearrangements in which the bithorax region is now at an ex-

treme distal location. These exceptions suggest that, again, somatic pairing begins proximally but that it may not reach completion in abnormally long chromosome arms relative to the timing of the trans-vection effect.

Somatic pairing as a causative or modifying factor in the position effect phenomenon has been often considered, as reviewed elsewhere (Lewis, 1950). Its detailed consideration by Ephrussi and Sutton (1944) warrants attention at this point. Thus, on their interpretation the forces of somatic pairing are assumed to cause a (reversible) deformation of the genes in the case of the structural heterozygote, rather than an alteration in the interaction of localized gene products. Their hypothesis, however, was designed for the general case in which there is an effect of a rearrangement on the wild-type allele of gene, *a*, such that R (+)/*a* differs phenotypically from +/*a*. The rearrangements with which the trans-vection effect deals do not show any effect on such heterozygotes in the case of either the bx^{34e} gene and its normal allele or the *Ubx* gene and its normal allele; nor is there a difference when bx^{34e} *Ubx*/+ + and bx^{34e} *Ubx*/R (+ +) are compared. Thus, the available evidence in the case of the trans-vection effect strongly implies that transport of gene products rather than deformation of the genes is involved.

Much more detailed studies will be needed before speculations are warranted regarding the manner in which the developmental changes seen in the trans-vection effect are mediated. But it should be noted that this type of position effect provides a possible new approach to studying gene action, since it specifies the conditions under which experimental variation in the strength of somatic pairing may *per se* cause a change in gene action.

The results of the application of the bithorax method to the measurement of rearrangement production will be only briefly discussed. It may be inferred from the data of Bauer and others (Bauer, 1939) that the present method detects roughly 10 per cent of the rearrangements that would be detectable by the salivary gland chromosome method (for example, at a dose of 3,000 r of X-rays, the present method recovered 2.3 per cent flies in grades 2–4, inclusive, while the cytological method showed that 18.8 per cent of the treated sperm carried rearrangements). This must be considered a very high yield of rearrangements for a position effect method and it is in all probability dependent upon the great length of the critical region. Thus, the present method has roughly ten times the efficiency of perhaps the next most efficient position effect method available; namely, that employing the Dubinin-effect, or position effect of the cubitus interruptus gene (inferred from the data of Khwostova and Gavrilova, 1938).

There is an indication from the present data that gamma rays may be slightly less effective than X-rays in producing chromosomal rearrangements in Drosophila but the point is by no means established (cf. similar findings with Tradescantia by Kirby-Smith and Daniels, 1953). It is safe to conclude, however, from the present data that fast (pile) neutrons are many more times effective than gamma rays or X-rays in producing rearrangements in this organism.

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SUMMARY

A new type of position effect called the "trans-vection effect" permits rapid and highly efficient detection of chromosomal rearrangements in the first generation following an induction treatment. Several unique features are involved: (1) the position effect extends over vastly greater distances than heretofore demonstrated (over 500 discs of the salivary gland chromosomes); (2) wholly euchromatic as well as euchromatic-heterochromatic rearrangements are efficiently detected; and (3) the position effect is detectable only in a double heterozygote between pseudoallelic mutant genes, the arrangement of which must be of the trans-type (a +/+ b). Interference in somatic pairing exerted by structural heterozygosity is postulated to reduce the transport of an essential gene product from one chromosome of this heterozygote to the other. By the use of this new method fast (pile) neutrons have been found to be more effective than X-rays or gamma rays in producing rearrangements in Drosophila, and estimates of the dose of fast neutrons at different stations during a nuclear detonation have been derived.

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VISIBLE AND LETHAL MUTATIONS IN DROSOPHILA[1]

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INTRODUCTION

Foremost among the questions confronting us in the investigation of the genetic effects of nuclear detonations is that concerning the relative biological effectiveness (RBE) of fast neutrons and X-rays. Contrary to most of the published reports on fast neutrons versus X-rays in producing mutations and chromosome alterations in Drosophila (Catcheside and Lea, 1945; Catsch *et al.*, 1944; Demerec *et al.*, 1942; Dempster, 1941; Eberhardt, 1943; Fano, 1941, 1944; Giles, 1943; Lea, 1947; Thoday and Lea, 1942; Timoféeff-Ressovsky and Zimmer, 1938, 1939; Zimmer and Timoféeff-Ressovsky, 1938, 1942), we find that fast neutrons are at least twice as effective or have a much higher RBE for all the genetic effects studied.

Several genetic criteria have been used to measure the degree of the effect caused by treating the mature male germ cells of Drosophila with X-rays and with fast neutrons both from nuclear test devices and from the cyclotron: the frequencies of (1) recessive visible mutations at specific loci on the third chromosome, (2) dominant visible mutations, both autosomal and sex-linked, (3) dominant Minute effects, and (4) recessive sex-linked lethal mutations. In addition to our own data we shall include some results of other investigators, notably those of Baker and Von Halle (1954) on dominant lethal effects. The most commonly used measure of radiation damage is the rate of sex-linked recessive lethal mutations because of the greater ease and objectivity of scoring as compared to most other types of effects. Recently, the use of dominant lethals has come into favor for testing the effectiveness of radiations (Pontecorvo, 1942; Lea and Catcheside, 1945; Baker and Von Halle, 1953; Lüning, 1952). It would be a mistake to restrict our observations on mutation frequencies to lethals, however, since there is evidence that they are not entirely analogous to visible gene mutations.

Most of the earlier studies on visible mutations have dealt with sex-linked characters because of the greater ease of detecting them (Timoféeff-

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Ressovsky, 1932, 1933, 1934; Johnson and Winchester, 1934; Timoféeff-Ressovsky and Delbrück, 1936; Muller, 1951; Valencia and Muller, 1949). Among the few investigators who have obtained information on the rate of mutations at specific loci on autosomes are Patterson (1932, and unpublished), Fryer and Gowen (1942), and Alexander (1952). This method of determining mutation rates at specific loci should offer one of the most precise means of estimating the relative effects of different types of radiation as well as of comparing the reactions of different genes to the same treatment. Its chief drawbacks are its more tedious mating procedures and the necessity of obtaining much larger numbers since the rates are lower than for lethals.

The first two papers of this symposium have dealt with chromosome breakage and rearrangement and, although this report is concerned with visible and lethal mutations, it cannot ignore chromosome breakage since many of the mutations are the result of or are associated with breaks in the chromosomes. This is true not only with the dominant visible effects, most of which are lethal when homozygous, but also of recessive lethals and visibles (Muller, 1940, estimated that about one-third are small deficiencies), and perhaps most of all of dominant lethals, which are known to be due almost entirely to chromosome breaks and asymmetrical rearrangements (Catcheside and Lea, 1945).

Obviously it is desirable to compare the effectiveness of different types of radiations in producing all these various genetic effects. From such comparisons we may be able to infer something about the mechanisms involved in producing the changes and perhaps something about the nature of the mutations themselves.

DOSIMETRY

Most of our uncertainties regarding dose-frequency relation and especially the relative biological effectiveness of fast neutrons as compared to X-rays rest upon our lack of precise knowledge of neutron dosage. It is necessary to subtract the reading of gamma-ray component from the total dose, and if this is a large fraction and not well known, the neutron dosage is uncertain. Since the biological effects of neutrons have RBE's considerably greater than 1, the presence of unknown gamma rays will cause an underestimation of RBE, or an overestimate of gamma contamination will result in a spuriously large RBE.

One possible error in neutron measurement in the nuclear detonations is that the duration of the neutron burst is so short that all of the ions formed might not be collected, causing the ionization dosimeters to give a low reading. If the biological effects are in proportion to the total neutrons present under these conditions rather than to the dosimeter reading, then RBE's based on the dosimeter readings are overestimated.

Neutron dosimetry presents many difficulties even with the cyclotron under relatively controlled laboratory conditions and especially in the field with nuclear test devices. Sheppard and Darden (1953) discuss the main

problems in fast neutron measurement.[3] Our readings on the Oak Ridge National Laboratory 86-inch cyclotron were made before each treatment with a specially calibrated dosimeter. The variation in neutron intensity in different parts of the facility was compensated for by shuffling the capsules containing the flies being irradiated. The gamma-ray contamination was estimated to be about 10.3 per cent of the dose. Estimates of the neutron spectrum indicate that only about 10 per cent of the neutrons have energies greater than 3 Mev, and that the population peak was about 1 Mev.

The dosimetry in the nuclear test devices was particularly difficult. The flies were placed in lucite tubes containing small amounts of cellucotton moistened with sterile sugar water, and the tubes were put inside 7-inch-thick lead hemisphere shields at successive distances from ground zero in each detonation used. Inside each hemisphere with the biological specimens were placed (1) film badge and chemical dosimeters designed to measure gamma radiation, (2) sulfur, gold, and manganese neutron detectors, and (3) other dosimeters designed to measure neutron radiation in rep (roentgen equivalent physical). Since each type of dosimeter shows some sensitivity to all types of radiation with some degree of energy dependence, the exact neutron rep value is extremely difficult to determine. The final values were arrived at by extrapolation from Sheppard's semitissue-equivalent measurements in a curve parallel to the sulfur-flux curve. From the sulfur and gold measurements it was concluded that the energy distributions in the nuclear detonations were about the same as in the cyclotron, i.e., a population peak of about 1 Mev. Gamma rays were effectively shielded out of the hemispheres by the 7-inch-thick lead walls, but considerable gamma contamination can originate in the walls themselves and contents of the hemispheres. The amount of gamma radiation has been estimated to be approximately 10–35 per cent depending on the station.

SPECIFIC LOCUS TESTS WITH THIRD CHROMOSOME MARKERS IN THE RES STOCK

Irradiated wild-type males (Oregon-R), from 2 to 4 days old, were mated to virgin *res* females and were transferred to fresh food bottles three times before being discarded. The males remained with females a maximum of 6 days after treatment, thus assuring that only postmeiotic germ cells were used in producing progeny. All F_1 flies exhibiting either partially or entirely any one or more of the mutant phenotypes were tested further in order to determine whether the variants should be classified as specific germinal mutations or as mimics, dominant mutations at other loci, or modifiers.

The rates of X-ray-induced mutations at individual loci range from 0.84 at *hairy* to 9.21 at *ebony-sooty*, while the average for all eight loci is $3.35 \times 10^{-4}/r$. Other studies of mutation rates have yielded figures which

[3]Since this symposium was presented Sheppard and Darden have explained the physical dose estimates in both cyclotron and nuclear detonation experiments (see Kirby-Smith and Swanson, 1954).

TABLE 1

MUTATION RATES AT SPECIFIC LOCI AND AVERAGE RATE FOR THIRD CHROMOSOME MARKERS

Source of Radiations	Dose	Sample size	Rates at specific loci per roentgen equivalent ($\times 10^{-6}$)								Average rate/rep ($\times 10^{-6}$)
			ru	*h*	*th*	*st*	*pp*	*cu*	*sr*	e^a	
X-ray	3000 r	39,823	(3)[a]	(1)	(2)	(3)	(2)	(3)	(7)	(11)	(32)
			2.51	0.84	1.67	2.51	1.67	2.51	5.86	9.21	3.35
Cyclotron	1000 rep	15,260	(2)	(2)	(1)	(0)	(3)	(3)	(1)	(3)	(15)
			13.11	13.11	6.55	....	19.66	19.66	6.55	19.66	12.29
Nuclear test devices	Various	21,670	(7)	(3)	(3)	(5)	(9)	(2)	(8)	(3)	(40)
			20.93	8.97	8.97	14.95	26.91	5.98	23.92	8.97	14.95
Control	....	18,802	....	....	....	....	....	....	....	....	0

[a]The figures in parentheses indicate the actual numbers of proved germinal mutants at each locus.

compare favorably with our own. Alexander (1952) obtained an average rate of $5.98 \times 10^{-6}/r$ for the same *res* markers, and Patterson's data (unpublished) gave an average of 3.4×10^{-6}. Neither of these figures is significantly different from our values.

Table 1 summarizes results obtained from the three types of experiments, cyclotron, X-ray, and nuclear test devices. Data from two X-ray experiments are combined and all data from the nuclear test devices are pooled and calculated on the basis of rate/rep/locus $\times 10^{-6}$. These figures have been used to calculate the relative biological effectiveness (RBE) of fast neutrons as compared to X-rays of 250 kvp, as shown in table 2.

In a comparison of the cyclotron effects with the X-ray rates we find a range of neutron effectiveness from 1.2 to 16.9 at different loci, with a mean RBE of 4.0. The RBE's from neutrons from the nuclear test devices as compared to X-rays range from 1.0 at the *ebony-sooty* locus to 16.1 at the *pink-peach* locus. The RBE for the average of all eight loci on the third chromosome is 4.5.

It is possible that the loci may differ in their response to radiations, but our data are insufficient to establish this; furthermore, since the variations

TABLE 2

ESTIMATED RELATIVE BIOLOGICAL EFFECTIVENESS (RBE) IN PRODUCING MUTATIONS AT SPECIFIC LOCI OF FAST NEUTRONS AS COMPARED TO 250 KVP OF X-RAYS

Source of radiations	Specific loci								Mean RBE
	ru	*h*	*th*	*st*	*pp*	*cu*	*sr*	e^a	
Cyclotron	5.6	16.9	4.1	....	12.9	8.5	1.2	2.3	4.0[a]
Nuclear test devices	8.3	10.7	5.4	6.0	16.1	2.4	4.1	1.0	4.5

[a]Corrected for 10 per cent gamma contamination.

in RBE's for different loci for cyclotron and nuclear detonations are not consistent, it may well be that they do not represent real differences. The average rates for all eight loci and the RBE's calculated from them give a much better comparison.

Since our data on specific loci mutations have been derived from one dosage level of X-rays (3000 r) and only one dosage level in the cyclotron (1000 rep), we cannot compute the RBE in the usual fashion of determining the dose of neutrons which will give equal biological effects to a given dose of X-rays and dividing the latter figure by the former; we can only compute the mutation rate per rep then divide the rate for neutrons by the rate for X-rays. This method is satisfactory if the different dose levels give straight-line curves in the range used in the experiments. The values might be quite different, however, if these curves converged at higher doses, for example.

DOMINANT VISIBLE MUTANTS (EXCLUDING MINUTES)

In the experiments designed for the study of specific loci mutations, all variants were recorded whether or not they resembled the *res* markers. Each variant was tested to determine if it behaved as an inherited dominant trait. The most common modifications encountered were rough eyes, Plexate wings (either with or without Minute effects), and Notch wings, although a number of other wing, eye, and bristle effects occurred. Table 3 summarizes the results of these tests.

These visible mutants usually are lethal when homozygous and nearly always are associated with structural changes of one kind or another. Some are very small deficiencies like *Notch*, others are minute duplications like *Hairy-wing*, and still others are small inversions like *Dichaete*. Some dominant visible mutants are associated with large inversions, e.g., *Curly*. It is to be expected that such a variety of effects, the loci of which are distributed over all the chromosomes, would not give a very consistent response to radiation. Furthermore, a large proportion of the "observed" variants were sterile or failed to breed true. Some of these cases could be mimics or developmental anomalies, but if correction is made for mimics on the basis of control rates, there remains a significant excess which must represent somatic mutations or dominant effects which can survive in the heterozygous condition in the F_1 individuals but which give rise to inviable combinations in the resulting gametes. We therefore have entered the total observed variants in table 3 and have calculated rates and RBE's (compared to X-rays) for them in order to compare with the figures obtained for only those variants which bred true for their respective characters. In both cases, despite the heterogeneity of effects included in this category of dominant visible mutants, the neutron effects are much more striking than those of X-rays when compared on a per-rep basis. Ives *et al.* (unpublished) agree that fast neutrons produce proportionately more dominant mutations than soft X-rays.

TABLE 3

DOMINANT VISIBLE MUTATIONS (EXCLUDING MINUTES)

Source of radiations	Dose (rep) (D)	Total flies examined (T)	Number of mutants		Rates ($\times 10^{-6}$)		RBE	
			Observed (O)	Proved (P)	O/DT	P/DT	Observed	Proved
X-ray I	3000 r	9,237	4	3	14.43	10.83		
X-ray II	3000 r	25,775	31	17	40.10	21.99		
Cyclotron I	1000	6,496	11	5	169.33	76.97		
Cyclotron II	1000	15,260	38	9	249.02	58.98		
Combined data		21,756	49	14	225.23	64.35	5.6–15.6	2.9–5.9
Nuclear test device A	3500	668	9	2	384.94	85.54		
	2500	682	13	3	762.46	175.95		
	2000	5,057	49	25	484.48	247.18		
	1400	4,343	42	21	690.77	345.38		
	1000	3,689	14	6	379.51	162.65		
Combined data		14,439	127	57	530.80	238.24	13.2–36.8	10.8–22.0
Nuclear test device B	2900	870	13	4	515.26	158.54		
	1100	6,361	17	4	242.96	57.17		
Combined data		7,231	30	8	315.12	84.03	7.9–21.8	3.8–7.8

In the cases of dominant visible mutations our comparisons again are made on rates per rep at only one dose level for X-rays and cyclotron neutrons, giving an RBE of 5.6–15.6 for all observed variants and 2.9–5.9 for the proved mutants. Data from the nuclear test device A gave RBE's of 13.2–36.8 for all observed mutants and 10.8–22.0 for proved. RBE's computed from results of nuclear test device B were 7.9–21.8 for observed and 3.8–7.8 for proved mutants. These high values for dominant visible mutants, although more variable, parallel those for dominant lethals and provide additional evidence that these mutations involve chromosome breakage.

DOMINANT MINUTE MUTATIONS

Dominant Minute mutations constitute a large proportion of the visible mutants resulting from the different radiations; consequently they have been scored as a separate category and used as a criterion for determining dosage relations. Apparently, the first report on the utilization of dominant Minutes in this fashion is that of Glass (1952) who states that the dosage curves for Minutes produced by X-rays seem to be linear in both males and females. This suggests that Minutes are one-hit effects.

Most Minutes are known to be small deficiencies which are phenotypically expressed when heterozygous and are lethal in the homozygous condition. In some cases the only effect of such a deficiency is the reduction in size of bristles; but often other phenotypic expressions accompany the bristle effects, such as rough eyes, extra or anastomosing wing veins (Plexate), spread wings, and other abnormalities. These more drastic and pleiotropic effects apparently are due to the larger deficiencies. Most Minutes exhibit reduced fertility in either or both sexes and frequently Minutes showing other effects have low viability and complete sterility. Table 4 summarizes the data on Minute mutations. We have scored entire Minutes separately from the partials, and all single bristle effects have been excluded. Analysis of these data on dominant Minute mutants also

TABLE 4

RATES OF DOMINANT MINUTES PRODUCED BY X RAYS AND FAST NEUTRONS AND THE RELATIVE BIOLOGICAL EFFECTIVENESS OF NEUTRONS

Source of radiations	Rates of mutations ($\times 10^{-3}$)			Relative biological effectiveness		
	P + E/DT	P/DT	E/DT	P + E	P	E
X ray I	213.71	47.49	166.22			
X ray II	144.84	28.45	116.39			
Cyclotron[a]	1421.43	154.41	1267.01	6.7–9.8	3.3–5.4	7.6–10.9
Nuclear test device A	981.83	80.28	901.73	4.6–6.8	1.7–2.8	5.4– 7.8
Nuclear test device B	1039.91	168.06	871.84	4.9–7.2	3.5–5.9	5.3– 7.5

[a] Corrected for 10 per cent gamma contamination. P, partial Minutes; E, entire Minutes; D, dose; T, total flies examined.

shows a marked increase in effectiveness of neutrons over X-rays; the RBE for partials being 1.7–5.9, for entire Minutes 5.2–7.8, and for both combined 4.6–9.8. Thus the fast neutrons prove to be much more efficient in producing deficiencies than X-rays.

Kaufmann (1941) reported that neutrons produced many deficiencies larger than those produced by X-rays, although he found them to be about equal in number. Since our Minutes have not been checked cytologically, we do not know the extent of the deficiencies, but the proportion which show pleiotropic effects and sterility would suggest a greater proportion of larger losses among neutron-induced Minutes.

The total rate which we obtained at 3000 r of X-rays is somewhat lower than that reported by Glass (1952), 0.63 per cent and 0.91 per cent, respectively. This difference might be explained on the basis of differences in stocks used; the viability of Minutes may have been reduced considerably in the flies heterozygous for the *res* markers in our experiments.

SEX-LINKED RECESSIVE LETHAL MUTATIONS

Irradiated wild-type males (Oregon-R), 2–4 days old, were mated to virgin Muller-5 females and were transferred to fresh food bottles three times before being discarded. Only those germ cells which were postmeiotic at the

TABLE 5

SUMMARY OF SEX-LINKED RECESSIVE LETHAL MUTATIONS INDUCED BY OAK RIDGE NATIONAL LABORATORY 86-INCH CYCLOTRON AT FOUR DIFFERENT DOSE LEVELS

Dose (rep)	Number of lethals	Chromosomes tested	Per cent lethal
500	69	2118	3.26
1000	91	1393	6.53
1500	100	1091	9.17
2000	57	418	13.64

time of treatment were used in producing progeny. Individual F_1 females were mated to Muller-5 males and the separate cultures examined for the presence of wild-type males. All cultures lacking such males and thus classed as containing lethals were tested for another generation to verify the classification. Table 5 shows the results of experiments with the cyclotron and table 6 the results of a nuclear detonation. Some data from Baker and Von Halle (unpublished) have been incorporated at three dosage levels. All these data are compared to those derived from X-ray experiments illustrated in fig. 1. It will be noted that the regression lines for lethals produced by the cyclotron and nuclear detonations are essentially parallel. Certainly there is no significant difference in the slope between the two in view of the degree of accuracy of our dosage measurements. On the other hand, the difference in slopes between these and the X-ray curve is striking, indicating an RBE of neutrons as compared to X-rays of about 2. This value is less than that observed for specific loci mutations and

TABLE 6

SUMMARY OF SEX-LINKED RECESSIVE LETHAL MUTATIONS INDUCED BY NUCLEAR TEST DEVICE B

Estimated total dose (rep)	Number of lethals	Chromosomes tested	Per cent lethal
2900	13	96	13.54
1100[a]	110	1918	5.74
640[a]	88	2808	3.13
420	28	1314	2.13
230[a]	7	546	1.28
Control	3	1339	0.22

[a]Some unpublished data contributed by Baker and Von Halle incorporated with our own at these dose levels.

for dominant visibles; it is much less than the RBE for dominant Minutes and for dominant lethals.

Unpublished data on sex-linked lethals from nuclear detonations were supplied by Drs. P. T. Ives, R. P. Levine, and H. T. Yost, and we appreciate their permission to compare our results with theirs. Table 7 shows

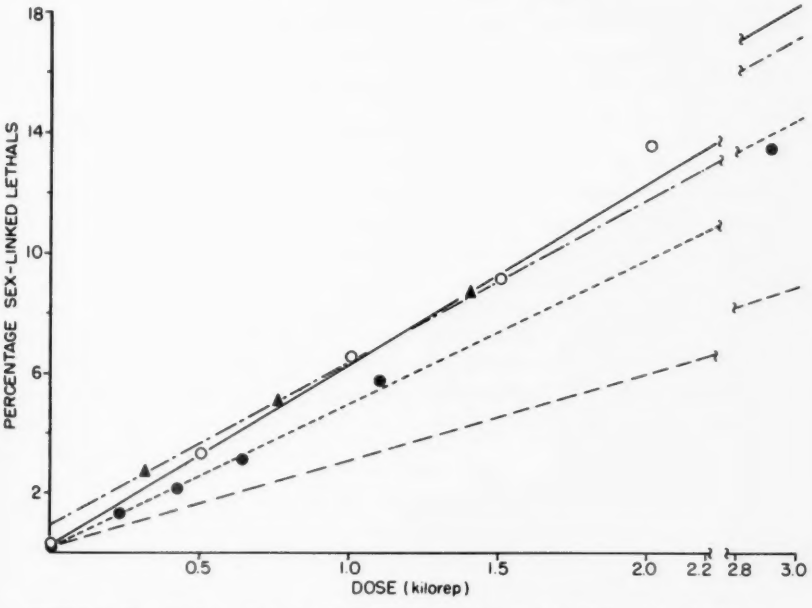

FIGURE 1. Rates of sex-linked recessive lethals induced by different radiations.

——— O = Oak Ridge National Laboratory 86-inch cyclotron
– – – – ▲ = Nuclear device A (Ives)
- - - - - - - - ● = Nuclear device B
— — — — = X-rays

the percentage of lethals obtained at four different doses and fig. 1 includes the regression line calculated from the data of Ives *et al.* Our values are similar but consistently lower. Ives suggests that fast neutrons from nuclear devices produce simple lethals (i.e., those not associated with chromosome aberrations, as determined by crossover tests) with an RBE of about 3 as compared with soft X-rays, but gross chromosomal aberrations show an RBE of about 15.

We are grateful also to Dr. J. W. Gowen, who has kindly permitted us to quote unpublished data from nuclear detonation experiments. He concludes that neutrons are of the order of two times more effective than X-rays in producing sex-linked lethal mutations. He states further that gene mutations are less likely to be induced by fast neutrons than are chromosome breaks, although they are more frequent from a given dose of neutrons than from a comparable dose of X-rays.

The results from numerous investigations using X-rays have shown that there is a linear relation between dose and frequency of sex-linked recessive lethal mutations. Herskowitz (1951) summarizes these studies and shows that the frequency increases linearly at the rate of 2.9 per cent/1000 r of X-rays. Our theoretical curve for X-ray-induced lethals on fig. 1 is based on this figure. Herskowitz concludes that these lethals have three origins: point mutation, 0.6 per cent; breakage alone, 2.0 per cent; and position effect following rearrangement, 0.3 per cent.

The difference between the rates of sex-linked recessive lethals obtained in cyclotron experiments and nuclear detonations were not significant when tested both by regression line and χ^2 tests; therefore, it is valid to group cyclotron and nuclear test device data. The results from both show a good fit when plotted by weighted linear regression; therefore, the RBE for different dose levels would remain the same. Our data indicate that the percentage of lethals induced by neutrons is between 5 and 6/1000 rep, which gives an RBE of about 2.

This differs from the reports appearing in the literature regarding the relative efficiency of fast neutrons and gamma rays in producing sex-linked lethals (Timoféeff-Ressovsky and Zimmer, 1938, 1939; Zimmer and Timoféeff-Ressovsky, 1938, 1942; Dempster, 1941; Giles, 1943; Fano,

TABLE 7

COMPARISON OF SEX-LINKED RECESSIVE LETHAL RATES OBTAINED IN DIFFERENT DETONATIONS

Dose (rep)	Nuclear test device A (Ives *et al.*) (per cent)	Nuclear test device B (Mickey and Yanders) (per cent)
315	2.66	1.71
760	5.08	3.83
1400	8.56	6.88
2500	14.55	12.12

1944; Demerec *et al.*, 1942; Fano and Demerec, 1941) all of which seemed to indicate that neutrons give a yield for equal ionization in the tissue of about two-thirds that for X-rays. A number of factors may combine to account for this discrepancy. Firstly, the dosimetry of fast neutrons is subject to wide variations, particularly in regard to the conversion factor necessary for different Victoreen instruments in converting the n units to rep. Secondly, the amount of gamma contamination in the cyclotron treatments may have been neglected, which of course would give an underestimation of the RBE. In our own experiments the gamma-ray component was known to be about 10 per cent of the total dose. Finally, since Giles and Conger (1950) have demonstrated that the efficiency of a given dose of fast neutron ionization in inducing chromosome interchanges is inversely related to the energy of the protons producing the ionization, we may suspect that the induction of sex-linked lethals is energy dependent also. If this is true, then our experiments might be expected to show a much greater effectiveness of neutrons because the energy distributions in the cyclotron were estimated to have a population peak of 1 Mev (less than 10 per cent over 3 Mev), and the spectrum from nuclear detonations must have been quite comparable.

One other puzzling aspect of the data for sex-linked lethals requires attention, namely, why should the RBE for sex-linked lethals be lower than that for specific loci mutations? Presumably, many of the latter are gene mutations not associated with breaks, although some are known to be small deletions and others to be position effects; while just as many if not more of the lethals involve breaks and deletions as the specific loci mutations. Chromosome breaks and rearrangements have been shown to be more effectively produced by neutrons than by X-rays (Giles, 1940, 1943; Gray, 1946), consequently, lethals would be expected to show a higher RBE than visibles. Two factors may help to explain why the frequency of lethals falls below expectation. Firstly, the dosage relation of sex-linked lethals at high dose is still linear in spite of the composite nature of effects involved (deletions, gene mutations, position effects). Muller (1950) points out that at higher doses the siphoning off of one-hit breaks into two-hit aberrations such as dominant lethals will tend to reduce the number of sex-linked recessive lethals which are due to small deletions. Secondly, the clustering phenomenon which Muller (1951) describes with neutrons would reduce the number of separately detectable lethals.

DOMINANT LETHAL EFFECTS

We are indebted to Dr. W. K. Baker and Mrs. E. S. Von Halle for permission to use data unpublished at the time of this symposium but subsequently published (see Baker and Von Halle, 1954) on dominant lethals resulting from cyclotron and nuclear detonation tests. These data are compared with those obtained from X-rays (Baker and Von Halle, 1953). Since dominant lethals cannot be maintained and studied in successive generations, they must be studied by the method of egg counts. Details of the experiment are described in the paper by Baker and Von Halle (1953).

Fig. 2 shows the percentage of eggs hatching plotted against dosage of 250 kvp of X-rays and cyclotron neutrons as well as nuclear-test-device neutrons. The RBE of cyclotron neutrons thus seems to be considerably greater than X-rays in producing dominant lethals; in the lower dosage range the RBE is about 7, whereas at the higher dosages it falls off to about 4.

Dominant lethals are almost entirely chromosome structural changes rather than gene changes (Muller, 1940; Catcheside, 1948). These are

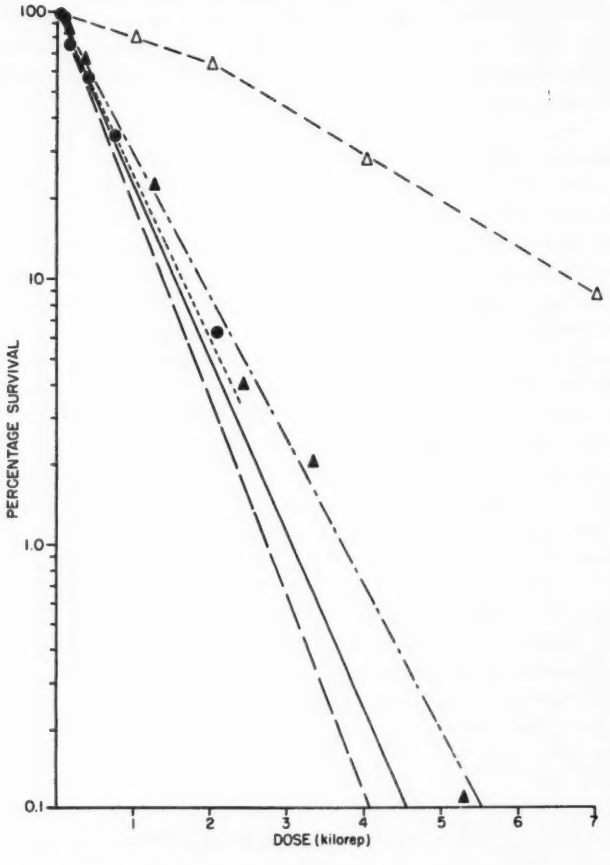

FIGURE 2. Rates of dominant lethals induced by different radiations. (From Baker and Von Halle.)

———— = Cyclotron
— — — ▲ = Nuclear device A
-------- ● = Nuclear device B
— — — Δ = X-rays
———— = Pure neutrons

constituted in part of large deficiencies and asymmetrical interchanges involving two or more breaks (Pontecorvo and Muller, 1941; Pontecorvo, 1942; Demerec and Fano, 1944; Catcheside and Lea, 1945) and in part of single breaks which fail either to restitute or to interchange, but instead undergo sister reunion.

The dominant lethals resulting from nuclear detonations are compared to those from cyclotron experiments in fig. 2. Slightly higher survival rates occur in the former. This difference might be attributed to more gamma contamination in the nuclear test devices than in the cyclotron. Actually, the difference appears not to be really significant but rather more likely to be a chance variation.

CONCLUSIONS

1. Fast neutrons from the Oak Ridge National Laboratory 86-inch cyclotron and from nuclear test devices (about 1 Mev) are approximately four times as efficient in producing specific loci mutations at the *res* markers in the third chromosome of *Drosophila melanogaster* as are X-rays of 250 kvp.

2. Dominant visible mutations are produced at a much higher rate per rep of neutrons than per r of X-rays, the RBE of neutrons for all observed variants being from 6 to 37 and for the proved dominant mutations from 3 to 22.

3. The relative biological effectiveness of fast neutrons as compared to X-rays in the production of dominant Minute mutations likewise is quite high, ranging from 5 to 11 for entire Minutes in different experiments.

4. Contrary to reports of previous investigators that fast neutrons are only about two-thirds as effective as X-rays in producing sex-linked recessive lethal mutations, we find the neutrons to have an RBE of 2. The discrepancy may be the result of a number of factors—to errors in dosimetry, particularly in conversion factors for converting n units to rep, to unsuspected or underestimated gamma contamination in the doses and, perhaps most important to probable energy dependence similar to that demonstrated by Giles and Conger for chromosome interchanges in Tradescantia, i.e., the biological effect for a given dose is inversely proportional to the energy of the fast neutrons used.

5. Dominant lethals are produced at a much higher frequency per rep of fast neutrons than per r of X-rays, the RBE at lower doses being about 7 and at higher doses falling off to about 4.

It appears, therefore, with all the biological criteria used to measure the genetic damage of radiations in Drosophila, that fast neutrons cause a much greater effect than do X-rays.

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THE EFFECT OF NEUTRONS ON THYMIC AND CIRCULATING LYMPHOCYTES IN THE MOUSE[1]

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INTRODUCTION

The demonstration that the biological effect of ionizing radiation increases with increasing ion-pair density along the path of the incident beam, together with the fact that certain tissues appear relatively sensitive to radiations with high ion-pair density, led to considerable interest in neutron radiation as a possible therapeutic agent (Zirkle and Lampe, 1938; Axelrod, Aebersold, and Lawrence, 1941; Stone and Larkin, 1942). The evaluation of the efficiency of neutron radiation in producing various biological effects, both acute and chronic, became increasingly important as new laboratory sources were developed and the probability of exposure to neutron radiation in the laboratory environment increased. Depending on its initial energy, the neutron can liberate energy in tissue through one of three principal reactions. High energy neutrons can enter into scattering reactions with hydrogen atoms, resulting in the ejection of the stripped hydrogen nucleus or proton. Low energy neutrons can undergo capture reactions with nitrogen, resulting in the emission of a proton (N^{14}, np, C^{14}), or they can be captured by hydrogen with the subsequent emission of a gamma photon (H^1, $n\gamma$, H^2). When the probability of these various reactions is considered, the principal method of energy release is seen to be the recoil-proton event for all neutrons with energies greater than a few electron volts. For neutrons with energies less than a few electron volts, the proton and gamma-producing capture events predominate and are approximately equal in absorbed energy in small animals such as the mouse. The proton, a heavy charged particle, has an ion-pair density along its path many times greater than that of the secondary electrons produced by X or gamma radiation. Thus, per erg of energy liberated in a tissue mass, the proton is expected to produce greater biological effect than the less densely ionizing rays.

The ion-pair density along the proton path decreases with increasing proton energy. The proton produced in the nitrogen-capture reaction has an energy of 6.2×10^5 electron volts. Recoil protons produced through the

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scattering reaction of neutrons and hydrogen possess about one-half the energy of the striking neutron. They may be expected to range in energy from a few electron volts for striking neutrons of comparable energy to well over a million electron volts for high-energy incident neutrons. Thus the biological efficiency, per rep, would theoretically be expected to change with variation in neutron energy, being large where the incident neutrons possess energies between about 10^2 to 10^6 electron volts, less for the slow or thermal neutrons entering into the nitrogen capture reaction, and even less along the major portion of the path of extremely energetic recoil protons produced by high-energy neutrons. However, it must be remembered that rep per incident neutron changes markedly with varying neutron energy.

Studies of the biological effect of neutron radiation began with the development of the first sources (Lawrence, 1937). A wide variety of materials have been exposed and numerous reactions and effects studied (Axelrod et al., 1941; Lawrence, 1937; Zirkle and Aebersold, 1936; Marshak, 1942; Aebersold and Lawrence, 1942). Many of the experiments have suffered from the fact that the neutron-measuring techniques were not sufficiently advanced to permit an accurate estimate of the dose received by the irradiated specimen. Estimates of dose have been based either on theoretical calculations of energy release resulting from assumed neutron-tissue nuclei reactions, or on measurements made with ionization chambers designed for electromagnetic radiations only. Accurate comparison of the various data obtained is not possible because of differences in physical conditions existing between the various sources and differences in dose-measuring techniques.

Nevertheless, several general conclusions can be drawn from the various studies done. As was pointed out by Zirkle at the time of the first laboratory neutron experiments, the relative biological potency of neutron radiation, compared with that of X or gamma radiation, differs for different responses and even for the same response in different species (Zirkle and Lampe, 1938). This has been adequately borne out in numerous studies done with various materials using the same or generally similar neutron radiation, where errors in dose measurement are not considered to have been sufficient to account for the differences in biological effect of the neutron radiation seen. The further fact that values for the biological efficiency of neutron radiation determined with lower organisms have not appeared to bear any constant relation to that determined from mammalian effects, such as mortality or hematological change, makes prediction of possible effects in large animals from these data for lower forms open to considerable question.

From the data of previous experiments it has not been possible to determine what variations in RBE are likely to occur with variation in neutron energy. Similar responses have not been observed following exposure to several neutron radiations known to differ appreciably in energy. Giles' observation that neutron effect, per dose unit, increased with a decrease in average neutron energy magnitude appears inadequate for quantitative use,

although it is one indication that neutron radiation producing low-energy recoil protons (and alpha particles through n, alpha reactions with boron) may be more effective than is a higher energy spectrum (Giles, 1943).

In a recent series of experiments using thermal neutrons from a suitable column in a nuclear reactor, Brennan has studied the mortality response in mice, while Harris has reported on determinations of the relative efficiency of neutron radiation using decrease in splenic and thymic weight of the mouse as the measure of biological effect (Brennan et al., 1952; Harris and Brennan, 1952). Brennan found a value for the ratio of X-ray to neutron dose to produce the effect of less than 2.4:1, while the data reported by Harris indicate a slightly lower figure (about 2:1) for the decrease in weight of the organs measured. In these instances, the energy of the proton resulting from the neutron radiation was quite precisely known, the reaction resulting in its emission being the capture of the thermal neutrons by nitrogen in tissue. Physical measurements appeared sufficiently accurate to separate the neutron component from that of the gamma radiation present in the exposure geometry and the effects of neutron and X radiation were shown to be additive in a separate mortality study using mice.

With the development of nuclear test devices, an additional source of neutron radiation became available for study. Certain of the biological work done using this source is considered of sufficient interest to warrent its general presentation at this time. This paper reports certain of the data obtained with mice. The decrease in the weight of the thymus and the decrease in the peripherally circulating lymphocytes were measured in animals exposed to a portion of the neutron radiation from nuclear devices. Since the neutron spectrum to which the animals were exposed differed from that used by Harris and Brennan, the opportunity existed to compare the same response (thymic weight decrease) in the same species under two different radiation conditions and to determine what, if any, difference in neutron efficiency occurred with the difference in neutron energy between the two sources.

The loss in thymic weight was considered to represent the loss in lymphoid cells from that organ, while the decrease in peripherally circulating lymphocytes was considered to parallel the loss of precursor cells from the lymphopoietic centers as well as reflecting the initial decrease in the small lymphocyte mass of the animal. Using Drosophila eggs and wheat seedlings, Zirkle demonstrated that identical neutron radiations differ in their effect on similar cells or tissues in the same species (Zirkle and Lampe, 1938). The measurements made on the mice in the present experiment afforded an opportunity to determine if similar differences would be seen for similar (if not morphologically identical) cells in a mammalian species.

EXPERIMENTAL METHOD

LAf_1 female mice 6 to 9 weeks of age were used throughout the experiment. Animals were exposed to either X-radiation from conventional sources or to a portion of the neutron radiation from nuclear devices. Two separate neutron experiments were conducted, and for each experiment, a parallel

TABLE 1

RESULTS OF FIRST EXPERIMENT

Type of radiation	Dose (rep)	Number of animals	Mean thymic weight (mg)	Per cent decrease
X-ray	Stressed controls	59	54.9 ± 3.0[a]	....
	84	29	44.9 ± 1.1	18.2
	112	29	40.9 ± 0.8	27.2
	168	29	29.9 ± 0.6	45.5
	224	30	24.2 ± 0.6	56.0
	336	15	17.1 ± 0.4	68.8
	392	15	14.1 ± 0.6	74.4
	448	15	13.6 ± 0.5	75.1
	504	15	11.4 ± 0.5	79.3
	560	15	8.9 ± 0.3	83.8
	784	12	6.6 ± 0.4	88.0
Neutron	Stressed controls	90	65.1 ± 2.7[b]	....
		90	63.7 ± 2.8[c]	
	17	30	43.1 ± 1.1	33.8
	31	30	30.2 ± 0.7	53.6
	60	30	20.9 ± 0.4	67.9
	60	30	19.0 ± 0.7	70.8
	95	30	9.4 ± 0.3	85.3
	110	30	6.8 ± 0.3	89.5

(a) Standard error of the mean.
(b) Control group for 17, 31, 60 and 110 rep doses.
(c) Control group for 95 rep dose.

X-ray control study was done to allow a direct comparison of the neutron and electromagnetic radiation effects. Neutron-exposed animals were placed behind shielding material to attenuate the accompanying gamma radiation.

Considering the effect of shielding substances which intervened between the mice and the source, the average energy of the neutrons reaching the animals was thought to be relatively low. While the majority were above thermal energy, they were definitely less energetic than those encountered in the initial cyclotron experiments. The average energy of the recoil protons produced was considered to be less than 10^8 electron volts, and neutron-hydrogen scattering reactions projecting protons with energies below 5×10^5 electron-volts were considered to have accounted for over 70 per cent of the energy liberated in the animal's body. Thus the specific ionization of the protons was undoubtedly greater than that seen with either the thermal neutron radiation employed by Brennan or the cyclotron neutron radiation used in the early experiments.

Dosimetry was done as follows: The energy release in tissue-equivalent material resulting from the neutron fluxes to which the animals were exposed was determined with ionization chambers constructed so as to be tissue equivalent in their reaction to a wide range of neutron energies and calibrated to permit the direct determination of the tissue rep dose within the exposure geometry. While these chambers were sensitive to both the

neutron and gamma radiation present, measurement of the gamma component was possible through the use of a specially-constructed chamber sensitive to the gamma radiation but almost completely insensitive to neutrons. Theory of construction of these chambers have been given by Failla and Rossi (1950). In each instance the dose due to neutron radiation only is given, the accompanying 20 per cent gamma component having been subtracted from the total chamber reading.

In the first experiment, the characteristics of the control X-radiation were 220 KVP, 25ma, HVL of the filtered beam 2.6 mm Cu, target-animal distance 100 cm and dose rate 12 r/min. Animals were exposed essentially in free air. In the second experiment, X-radiation characteristics were 250 KVP, 15 ma, HVL of the filtered beam 1.5 mm Cu, target-animal dis-

TABLE 2

RESULTS OF SECOND EXPERIMENT

Type of radiation	Dose (rep)	Number of animals	Mean thymic weight (mg)	Per cent decrease	Abs. lymphocyte count (cells/mm³)	Per cent decrease
X-ray	Stressed controls	35	81.8 ± 2.1[a]	....	....	....
	56	20	65.7 ± 3.9	19.7	....	....
	112	20	46.3 ± 2.9	43.4	....	....
	168	20	36.3 ± 2.1	55.6	....	....
	224	20	33.2 ± 1.3	59.4	....	....
	280	20	30.5 ± 1.3	62.7	....	....
	336	20	21.5 ± 1.1	73.7	....	....
	448	20	27.3 ± 0.8	66.6	....	....
	672	20	15.7 ± 0.5	80.8	....	....
	896	19	9.7 ± 0.3	88.1	....	....
	Stressed controls	55	61.4 ± 2.9	....	6870	....
	34	27	61.4 ± 1.6	12.4	3840	44.1
	67	27	49.0 ± 2.5	20.2	3520	48.8
	133	28	33.2 ± 1.2	46.0	2490	63.8
	200	28	27.9 ± 0.8	54.6	1880	72.6
	267	27	24.3 ± 0.7	60.4	1480	78.5
	336	28	19.6 ± 1.0	68.2	1060	84.6
	400	28	16.1 ± 0.8	73.8	810	88.2
Neutron	Stressed controls	87	86.7 ± 1.7	....	6670	....
	8.5	30	56.2 ± 1.7	35.2	4030	39.6
	9.3	30	54.5 ± 2.2	37.1	4370	34.5
	11.0	29	50.0 ± 2.0	42.3	3130	53.1
	13.1	29	47.4 ± 2.5	45.3	2960	55.6
	Stressed controls	90	75.1 ± 1.2	....	8580	....
	9.9	28	50.0 ± 2.1	33.4	4275	50.2
	19.9	28	36.1 ± 2.0	51.9	4475	47.8
	26.0	25	41.6 ± 2.1	44.6	4820	43.8
	34.0	30	30.2 ± 2.1	59.8	3689	57.0
	52.0	30	24.1 ± 1.0	67.9	2410	71.9
	72.0	28	20.3 ± 1.2	73.0	1990	76.8

(a) Standard error of the mean.

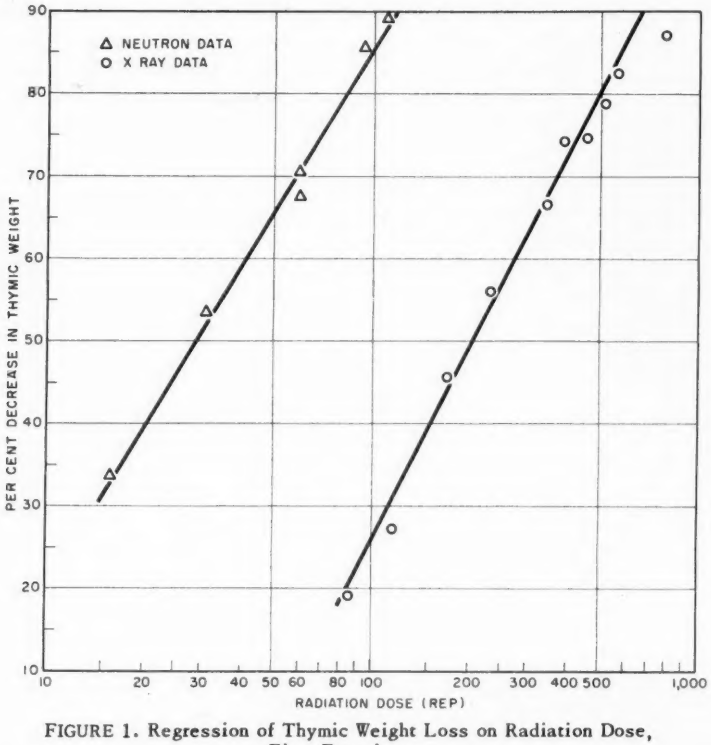

FIGURE 1. Regression of Thymic Weight Loss on Radiation Dose, First Experiment.

tance 100 cm and dose-rate 25 r/min. Scattering material was placed behind the animals, and the dose measured at the same point with the scattering material in position. In each experiment, the tissue dose, in rep, received by the animals was assumed to be 1.12 times the air dose recorded in roentgens with an ionization chamber.

Biological measurements included determination of the thymic weight and the absolute lymphocyte count in the peripherally-circulating blood. The later determination was carried out on certain animals in the second experiment only. Where only thymic weight was determined, animals were sacrificed with ether, and the thymus of each animal was removed and fixed in 10 per cent neutral formalin for 24 hours. At the end of this period of fixation, adherent mediastinal fat and connective tissue was dissected free, the organ blotted dry and weighed. Animals on which blood counts were done were sacrificed by decapitation. Blood was collected in siliconed watch glasses, and a single diluting pipette was used for each animal on which a total white cell count was made. Cells in both sides of the counting chamber were enumerated. Differential counts were made on coverslip smears stained with Wright's stain. Counting was continued

on each preparation until 100 cells had been examined or until 10 minutes had passed, whichever occurred first. The absolute lymphocyte count was taken to be the total white blood cell count times the per cent lymphocytes found on the differential enumeration.

The mean thymic weight or mean absolute lymphocyte count was determined for each exposed group of animals. The per cent decrease in either thymic weight or lymphocyte count was calculated for each group from appropriate control values, and regressions of the per cent decrease from normal values on radiation dose were calculated by conventional methods.

EXPERIMENTAL RESULTS

Data from the first experiment are summarized in table 1, while the comparable data from the second experiment are given in table 2. Doses given are either rep of X radiation in the case of the control animals, or the best estimate dose, in rep, for the neutron radiation determined by the methods given above. The data are graphed in figures 1, 2 and 3. The curves given are the calculated best lines of fit to the various points. Collected parameters of the

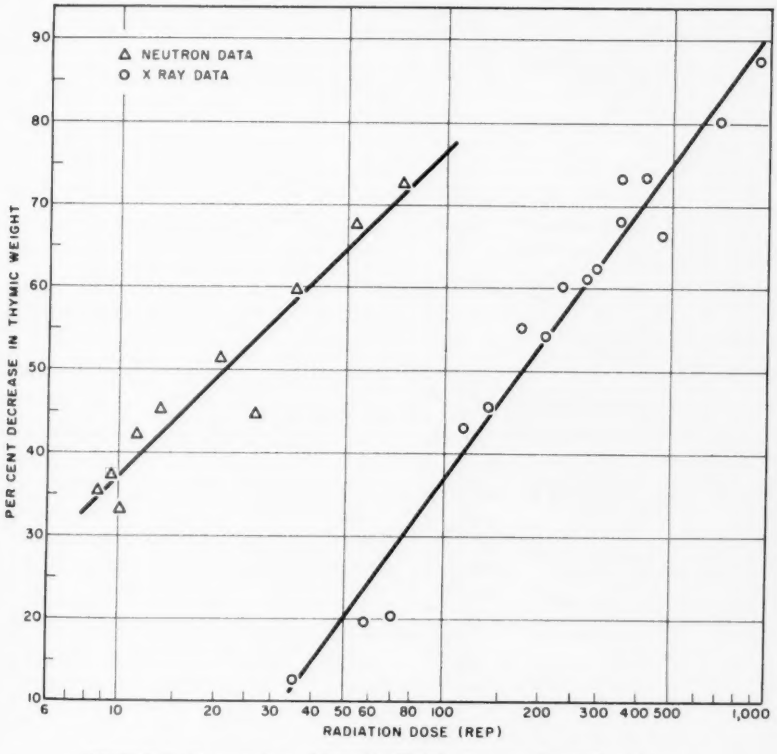

FIGURE 2. Regression of Thymic Weight loss on Radiation Dose, Second Experiment.

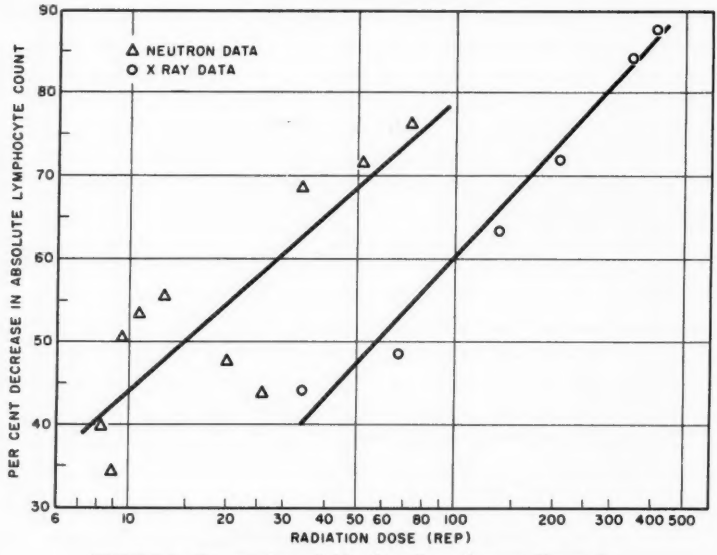

FIGURE 3. Regression of Absolute Lymphocyte Count decrease on Radiation Dose, Second Experiment.

various equations are presented in table 3.

The ratio between the dose of X radiation and neutron radiation required to produce a 50 per cent reduction in either thymic weight or absolute lymphocyte count was determined for each endpoint and each experiment. On the basis of thymic weight loss, neutron radiation was 7.4 times more effective than X radiation in the case of Experiment 1 and 8.1 times more effec-

TABLE 3

SUMMARY OF BEST LINES OF FIT TO THE BIOLOGICAL DATA*

Experiment	Biological response	Radiation	a	s_a	b	s_b	s^2
1	Thymus	X-ray	−124.08	4.89	75.21	3.38	9.53
		neutron	− 45.85	5.66	65.86	2.67	3.50
2	Thymus	X-ray	− 74.45	6.47	55.96	1.84	17.47
		neutron	− 2.31	5.51	39.90	4.16	17.15
	Lymphocyte count	X-ray	− 26.22	4.32	43.31	3.14	8.38
		neutron	8.26	10.96	35.69	8.29	67.99

*Assuming a relation of the form

$$y = a + b(\log x)$$

where y is the per cent decrease in thymic weight or absolute lymphocyte count, and x is the radiation dose in rep.

tive in the case of Experiment 2. On the basis of decrease in absolute lymphocyte count, the neutron radiation in Experiment 2 was approximately 3.9 times more effective than was the control X radiation.

A significant difference between the slope of the X ray and the neutron regression line for each of the paired experiments was seen in only one instance. This was the thymic weight decrease on the second experiment. With this one exception, it appeared that the effect of neutron and X radiation was qualitatively similar for each of the endpoints measured. The difference between the slopes of the X-ray curves on the first and second experiments was highly significant, as was the difference between the slopes for the corresponding curves for the neutron-exposed animals. These differences are thought to result from the fact that the two experiments were widely separated in time and location, and that the animal population in each instance was not strictly the same.

DISCUSSION

The neutron dose reported for each group of animals is considered to be the minimum tissue dose received. The values do not include the gamma radiation which reached the animals, while the biological effects recorded resulted from both the neutron radiation and the accompanying gamma component. While it is assumed that the additional biological response due to an added 20 per cent dose in rep from gamma radiation would not have been large, no attempt was made to subtract that effect from the total biological response seen since information on possible synergistic action of the two radiations was not available for these indicators. In addition it was possible that some saturation effect occurred in the tissue-equivalent ionization chambers with the neutron dose rates existing in the exposure geometry. This saturation, if it occurred, would make the recorded rep values lower than the actual levels which existed. This effect, combined with the fact that the biological responses included some gamma radiation effect, makes the ratios for biological effectiveness seen maximum for the indicators used and the neutron spectrum encountered. It seems unlikely that the combined effects of ionization-chamber saturation and gamma radiation would reduce the ratios seen by more than 30 per cent. However, a reliable estimate of the lower limits for the ratios is not possible until these questions are thoroughly investigated.

The majority of the neutron dose received by the animals in this experiment resulted from protons producing maximum or near-maximum specific ionization, about 2000 ion pairs per micron path length in tissue (Gray, 1950). The ratios of biological effectiveness observed using thymic weight loss, 7.4 : 1 and 8.1 : 1 can be compared with the value of approximately 2 : 1 derived from the data reported by Harris for the same indicator and for protons produced in the nitrogen-capture reaction of thermal neutrons. These latter protons have a specific ionization of nearly 1300 ion pairs per micron path length. Thus a 1.6-fold increase in specific ionization was accompanied by not more than a fourfold increase in biological effectiveness. Fur-

ther, it is considered that the ratios seen in this experiment represent the highest values which could occur for the particular effects studied with the neutron and X-radiations employed.

The ratio of biological effectiveness of neutron radiation determined by decrease in the peripheral lymphocyte count was half that observed for the decrease in thymic weight. Thus the fact that neutron radiation can exhibit significantly different quantitative effects on similar tissues in the same species is borne out for the small mammal as well as for the lower organisms tested by Zirkle (Zirkle and Lampe, 1938), and the *in vitro* lymphocytes from different sources tested with X radiation by Trowell (1952). As was pointed out by Stone (1948) these differences make values determined for one effect of little value in predicting other effects in the same or different species.

SUMMARY

The biological effectiveness of a portion of the neutron spectrum from nuclear devices, compared with that of X radiation, was determined using decrease in thymic weight and decrease in the lymphocyte count in the peripheral blood as the indicators of tissue damage. Comparing the ratios of biological effectiveness observed using thymic weight loss with similar data reported previously for thermal neutron radiation indicated that a 1.6-fold increase in proton specific ionization was accompanied by no more than a fourfold increase in biological effect. The neutron radiation employed was less effective in decreasing the lymphocyte count in the peripheral blood than it was in producing thymic weight loss, indicating that ratios for neutron effectiveness can differ for similar tissues in the small mammal as well as in lower organisms used in previous experiments.

ACKNOWLEDGEMENTS

The authors are particularly indebted to Drs. W. H. Langham, P. S. Harris and M. C. Fishler for their many helpful suggestions and participation in various portions of the experiments reported, as well as to Drs. C. W. Sheppard and H. H. Rossi for making available certain of their data used in the determination of the neutron tissue doses reported.

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THE RELATIVE EFFECTIVENESS OF NEUTRONS FROM A NUCLEAR DETONATION AND FROM A CYCLOTRON IN INDUCING DOMINANT LETHALS IN THE MOUSE[1]

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INTRODUCTION

Experiments on the genetic effects of ionizing radiation in mice are likely to be more accurate, and less expensive, if the animals are exposed to laboratory sources of radiation rather than to nuclear detonations. However, there are some features of the initial ionizing radiations from nuclear detonations that are difficult or impossible to duplicate in laboratory experiments. Among those not adequately tested for their biological effects, at the time when we were invited to participate in a field operation, were the special characteristics of the neutron radiation. The neutron radiation from a nuclear detonation differs from that produced in conventional laboratory sources principally in its intensity and energy spectrum. The energy spectrum of the neutron radiation, at a given point, from a nuclear detonation might be approximately matched in a laboratory experiment, but since the spectrum is difficult to measure, and since conventional laboratory sources cannot, in any case, produce the desired spectrum at the high momentary intensity of detonation neutrons, field tests of biological effects are necessary. The information obtained from such tests will be important in the estimation of human hazards under circumstances in which neutrons comprise a significant part of the total radiation. The proportion of neutron radiation in the total dose of ionizing radiation received from a detonation by an organism at a given place depends on many factors. All that need be said here is that the proportion may, under some conditions, be large enough to produce an appreciable part of the biological effect. This can be true, with biological responses for which neutrons have a high relative effectiveness, even when the neutron component of the total dose is much lower than the gamma component.

The main purpose of the present investigation was to attempt to discover, for the induction of dominant lethals in mice, whether or not the high intensity and the energy spectra, under the experimental conditions at various distances, of the neutron radiation from a particular nuclear detonation would show a biological effectiveness significantly different from that obtained in an earlier experiment with neutrons from a cyclotron, or from that to be obtained in anticipated experiments with neutrons from other laboratory sources. Dominant lethals were chosen for study because it was felt

[1]Work performed under Contract No. W-7405-Eng-26 for the U. S. Atomic Energy Commission.

that, with the limited number of animals that could be exposed, this particular effect would yield the most rapid and reliable answer to the above question so far as genetic damage in a mammal is concerned.

MATERIALS AND METHODS

The measurement of dominant lethality in the offspring of irradiated males is less complicated than in the offspring of irradiated females (Russell, L. B., and Major, 1953) and was the method chosen here. When male mice are exposed to a dose of radiation lying within most of the range of doses suitable for the study of dominant lethals, they remain fertile for a period of not longer than about four weeks or the time required to exhaust the supply of spermatozoa and maturing germ cells present at the time of irradiation. The sterility following this period is only temporary, fertility returning after an interval the length of which depends on dose and corresponds to the time required for the depleted spermatogonia to repopulate the seminiferous tubules. Dominant lethality in the offspring is high only for matings made during the initial fertile period, and it is high enough for these matings to give reliable estimates with only a small number of irradiated males. The results reported here are drawn solely from this mating period.

There has long been evidence, reviewed by Russell (1954), that most of this type of lethality results from chromosomal damage in the spermatozoa and, for later matings within the initial fertile period, possibly spermatids and spermatocytes. The motility and fertilizing capacity of the sperm are not affected, but when a sperm damaged in this way fertilizes an egg, the cleavage divisions are abnormal and the embryo dies, usually before or shortly after implantation. The effect has often been measured simply by recording the reduction in litter size in litters born to females mated with the exposed males. More accurate and informative data are obtained by the procedure, used here, of opening the females at a late stage in pregnancy and counting living and dead embryos, resorption sites and corpora lutea.

In order to reduce the gamma component of the radiation to a proportion that would not appreciably interfere with the estimation of neutron effects, the animals were shielded with lead. The exposure chambers available were lead hemispheres of 7-inch wall thickness and 14-inch inside diameter. The mice were confined inside these in round tubes 10¾ inches long, and 2 inches outside diameter, made of perforated "2S" (commercially pure) aluminum 0.032 inches thick. Six mice were placed in each tube.

The experimental material consisted of 144 young adult hybrid males obtained by crossing inbred 101 strain females with inbred C3H strain males. Twelve males were exposed in each of ten hemispheres placed at various distances from the detonation. The remaining 24 animals, used as controls, were placed in hemispheres two days before the detonation and for a length of time approximately the same as that required for the exposed animals. One day and a half after the detonation each male was placed with four adult untreated females of the same hybrid strain. Matings were detected by daily examination of all females for vaginal plugs. The uteri of

all females that were pregnant from matings made from two to six days after irradiation of the male were removed at a late stage in pregnancy (usually day 16½ or 17½) and the uterine contents examined under a dissecting microscope. Living and dead embryos were recorded and dead embryos were classified into two groups, (a) those which died between implantation and day 10½, and (b) those which died after day 10½, the classification being based on the external morphology of the embryo or, when the degree of disintegration made this impossible, on the size and appearance of the resorbing body. At the same time, the number of corpora lutea in the ovaries of each of the dissected females was recorded. At 18½ days after irradiation each surviving male was placed with a new group of four females. Pregnancies obtained from matings made from 19 to 31 days after irradiation were examined by the procedure used for the earlier pregnancies.

RESULTS AND CONCLUSIONS

Although the incidence of dominant lethality in the offspring of irradiated males is high for all matings made within the initial fertile period following exposure, the incidence varies to some extent according to the length of time, and probably the number of copulations, intervening between irradiation and the mating from which the dominant lethality is determined. Specifically, it has been clearly demonstrated (see review by Russell, 1954) that the incidence is higher for late matings than for early matings within the initial fertile period. The difference is usually explained by assuming that later matings utilize spermatozoa that were spermatids, or spermatocytes, at the time of irradiation, and by assuming, further, that these prespermatozoal stages are more sensitive. Whatever the cause is, the interval between the times of irradiation and mating must be considered in comparing the results of different experiments. It also seems likely that comparisons made on the basis of early matings will be less variable than those based on later matings, because all, or nearly all, the spermatozoa used in early matings will presumably have been irradiated as spermatozoa, whereas the spermatozoa used in later matings will probably be a variable mixture of cells irradiated at various late stages in spermatogenesis. For this reason, the more critical quantitative comparisons of nuclear detonation and cyclotron results to be considered here will be based on the early matings, namely, those made from two to six days after irradiation.

The results obtained from early matings of males exposed to the nuclear detonation are given in table 1 and figure 1. The dose estimates will be explained later. The decrease in number of pregnancies with increasing dose, for the higher doses, was the result of an increase in radiation sickness, or death during the mating period, of the exposed males. Instruments suitable for measurement of total dose at the three stations closest to the detonation were not available. The doses at these stations are estimated by extrapolation. The dominant lethal effect is summarized in the last column of this and other tables. It is expressed in terms of survival through

TABLE 1

DOMINANT LETHALITY IN OFFSPRING OF MALE MICE MATED FROM TWO TO SIX DAYS AFTER EXPOSURE TO NUCLEAR DETONATION

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
						Percentage of corpora lutea represented by:				
Exposure station	Total, neutron-gamma, dose. (Ion chamber readings)	Minimum estimate of dose of gamma radiation[a]	Maximum estimate of dose of gamma radiation[a]	Number of pregnancies	Number of corpora lutea	Living fetuses	Embryos or fetuses dying after day 10½	Embryos dying between implantation and day 10½	Eggs or embryos dying before implantation (100 minus sum of columns 7, 8 and 9)	"Survivors" (sum of columns 7 and 8)
	(rep)	(r)	(r)							
Control	0	0	0	48	446	84.30	0.45	3.59	11.66	84.75
Various	22	0.8	11	21	201	73.13	2.49	7.46	16.92	75.62
stations	50	1.5	22	22	208	60.58	0.48	19.71	19.23	61.06
arranged	83[b]	2	33	22	214	50.47	0.47	21.03	28.04	50.93
in order of	96	2.5	47	25	249	42.57	0.00	26.10	31.33	42.57
decreasing	131	3	62	21	198	34.85	0.00	27.78	37.37	34.85
distance	157[b]	4	67	24	232[d]	32.82	1.30	25.91	39.97	34.12
from	266.5[b]	5	83	17	155[d]	23.22	0.00	24.51	52.28	23.22
detonation	(322)[c]	6	120	8	80[d]	8.74	2.50	21.23	67.53	11.24
	(518)[c]	9	266	6	57[e]	0.00	0.00	13.99	86.01	0.00
	(761)[c]	11	426	2	19[e]	0.00	0.00	10.49	89.51	0.00

[a]See text for explanation.

[b]Mean of two readings.

[c]Estimated by extrapolation.

[d]Includes one or two pregnancies in which, because of early death of all embryos, there were no corpora lutea. In these cases the number of ovulated eggs was taken as 9.53, which is the mean number of corpora lutea per pregnancy for all pregnancies in which the corpora lutea were counted.

[e]There were no corpora lutea in any of these pregnancies. The number of ovulated eggs was estimated in the way described in footnote "d."

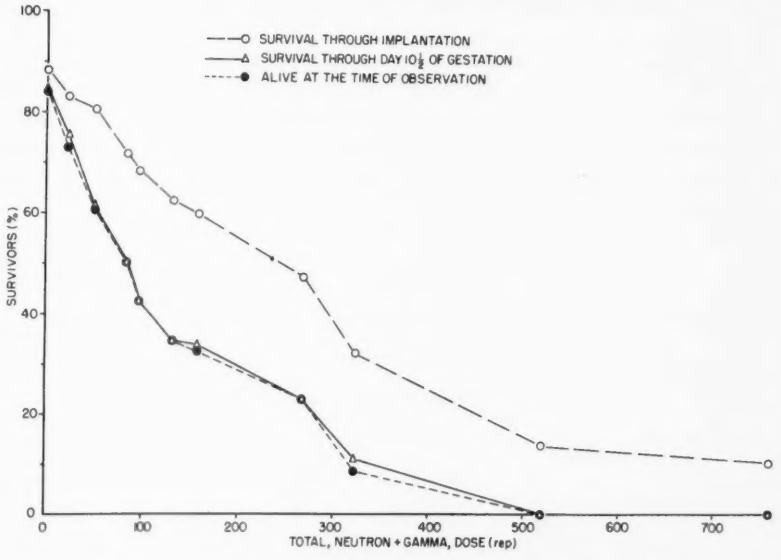

FIGURE 1. Dominant lethality in offspring of male mice mated from 2 to 6 days after exposure to nuclear detonation. Data from table 1.

day 10½ of gestation, since there is no statistically significant effect of radiation on mortality between this time and the time of observation.

Table 2 and figure 2 show the results obtained from a comparable cyclotron experiment (Russell, Russell, Gower and Sheppard, 1953). In this experiment, males of the same strain of mice were exposed in a lead chamber of 2-inch wall thickness to fast neutrons from a beryllium target placed in the proton beam of the Oak Ridge National Laboratory 86-inch cyclotron. The Victoreen dosimeter used was calibrated against three "tissue-equivalent" ion chambers. Gamma-ray contamination was estimated by a bismuth ion chamber to be approximately 10 per cent of the total dose in rep. Details of dosimetry are given by Sheppard and Darden (1953). The dose estimates cannot be regarded as precise, but they are presumably at least as reliable as those obtained in the detonation experiment.

Before discussing the results, it should be pointed out that the dominant lethal effect at high doses may be underestimated. This is more likely when litter size is used as the criterion of measurement, because some pregnancies will fail to produce any young at term, and it may not be possible, or easy, to distinguish these pregnancies from infertile matings. Such cases are detected by the procedure used here, of opening all females that have mated, provided at least one embryo survives through implantation. There may have been a few cases, at the higher doses, however, in which all embryos died before implantation and which were not, therefore, distinguished from infertile matings. This source of error cannot be

large, because the combined proportion of matings that were infertile and matings that were indistinguishable from this category, was not large at any dose. In any case, the data from the detonation for the three closest stations, at which there were no actual dose measurements, are excluded from the quantitative comparisons to be made between the detonation and cyclotron results. Furthermore, since any error for the remaining levels of effect would apply to both sets of data, it may be disregarded in these comparisons.

TABLE 2

DOMINANT LETHALITY IN OFFSPRING OF MALE MICE MATED FROM TWO TO SIX DAYS AFTER EXPOSURE IN CYCLOTRON

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
			Percentage of corpora lutea represented by:				
Dose (rep)	Number of pregnancies	Number of corpora lutea	Living fetuses	Embryos or fetuses dying after day 10½	Embryos dying between implantation and day 10½	Eggs or embryos dying before implantation (100 minus sum of columns 4, 5 and 6)	"Survivors" (sum of columns 4 and 5)
0	20	192	79.69	2.60	6.77	10.94	82.29
47	10	92	53.26	1.09	20.65	25.00	54.35
56	13	125	48.80	0.80	25.60	24.80	49.60
82	12	125	44.00	0.00	19.20	36.80	44.00
99	10	92	40.22	0.00	27.17	32.61	40.22
116	8	77	36.36	0.00	28.57	35.06	36.36
141	5	45	22.22	0.00	40.00	37.78	22.22
160	10	93.7[a]	25.61	2.13	33.08	39.17	27.75
194	6	66	27.27	1.52	21.21	50.00	28.79
209	5	52.7[a]	22.77	0.00	28.46	48.77	22.77
252	4	38.7[a]	10.34	0.00	25.84	63.82	10.34
257	2	20	10.00	5.00	45.00	40.00	15.00
305	1	9	11.11	0.00	22.22	66.67	11.11
311	1	9.7[a]	0.00	0.00	30.93	69.07	0.00
369	0	....	....	....	....	....	....

[a]Includes one pregnancy in which there were no corpora lutea owing to early death of all embryos. In this case the number of ovulated eggs was taken as 9.7 which is the mean number of corpora lutea per pregnancy for all pregnancies in which the corpora lutea were counted.

Various features of the cyclotron and detonation results can be compared. One of these is the distribution of ages, at death, of the affected embryos. First, it is apparent from tables 1 and 2, or figures 1 and 2, that, in both sets of data, death after day 10½ of gestation is negligible. Second, although the relative percentages of death occurring before and after implantation vary with dose, they are not significantly different in the cyclotron and detonation results for comparable levels of total effect. It is clear, then, that so far as distribution of deaths with respect to age

of embryos is concerned, there is no evidence of a qualitative difference in end results of the two experiments.

Another feature of the results on the basis of which the two experiments can be compared is the change in incidence of dominant lethality with

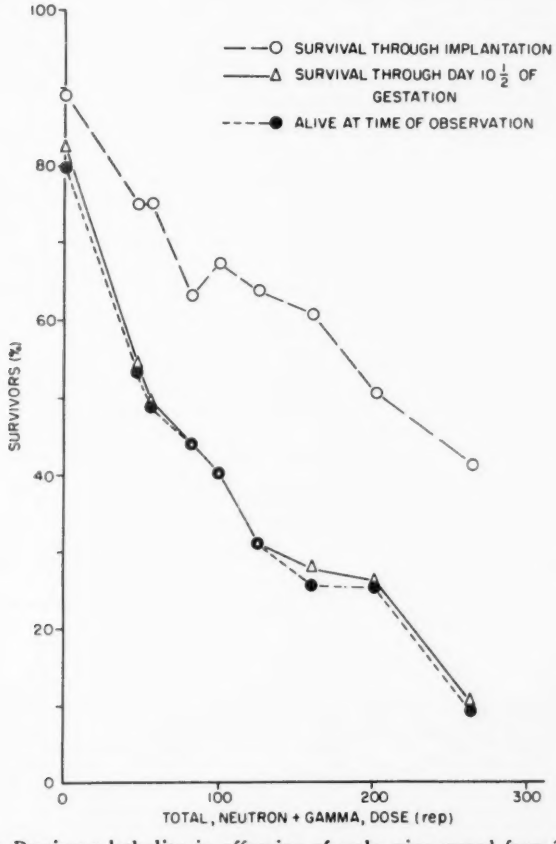

FIGURE 2. Dominant lethality in offspring of male mice mated from 2 to 6 days after exposure in cyclotron. Data from table 2. At some of the higher doses, where the number of pregnancies is small, the results for similar doses have been combined and plotted at the weighted mean dose.

time of mating after irradiation. Tables 3 and 4 show the results for the later mating period in the detonation experiment. This period is divided into two time intervals to show the change in incidence of dominant lethality occurring within it. Figure 3 shows the effect of time of mating on incidence of dominant lethality for all three mating periods. The incidence increases with time after irradiation for all doses except the lowest two

TABLE 3

DOMINANT LETHALITY IN OFFSPRING OF MALE MICE MATED FROM 19 TO 23 DAYS AFTER EXPOSURE TO NUCLEAR DETONATION

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
			Percentage of corpora lutea represented by:				
Total dose (rep)	Number of pregnancies	Number of corpora lutea	Living fetuses	Embryos or fetuses dying after day 10½	Embryos dying between implantation and day 10½	Eggs or embryos dying before implantation (100 minus sum of columns 4, 5 and 6)	"Survivors" (sum of columns 4 and 5)
0	21	199	79.90	1.51	5.53	13.07	81.41
22	17	159	61.01	1.26	18.24	19.50	62.26
50	24	225	53.33	0.00	27.56	19.11	53.33
83	12	111	33.33	0.00	32.43	34.23	33.33
96	18	160[a]	24.32	1.25	36.79	37.65	25.56
131	17	151[a]	18.52	1.32	35.72	44.44	19.84
157	15	134[a]	11.16	0.00	38.70	50.14	11.16
266.5	4	26[a]	3.79	0.00	30.33	65.88	3.79

[a]Includes one or two pregnancies in which, because of early death of all embryos, there were no corpora lutea. In these cases the number of ovulated eggs was taken as 9.19, which is the mean number of corpora lutea per pregnancy for all pregnancies in which the corpora lutea were counted.

TABLE 4

DOMINANT LETHALITY IN OFFSPRING OF MALE MICE MATED FROM 24 TO 31 DAYS AFTER EXPOSURE TO NUCLEAR DETONATION

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
			Percentage of corpora lutea represented by:				
Total dose (rep)	Number of pregnancies	Number of corpora lutea	Living fetuses	Embryos or fetuses dying after day 10½	Embryos dying between implantation and day 10½	Eggs or embryos dying before implantation (100 minus sum of columns 4, 5 and 6)	"Survivors" (sum of columns 4 and 5)
0	31	270[b]	75.56	1.48	6.67	16.29	77.04
22	12	105	79.05	0.95	7.62	12.38	80.00
50	11	105[b]	53.34	2.86	13.34	30.47	56.20
83	12	110[b]	22.73	0.00	19.10	58.17	22.73
96	6	57[b]	17.55	0.00	12.28	70.17	17.55
131	5	45[b]	8.90	0.00	28.92	62.19	8.90
157	5	45[c]	0.00	0.00	22.26	77.74	0.00
266.5	1	9[c]	0.00	0.00	22.26	77.74	0.00
(322)[a]	1	9[c]	0.00	0.00	11.13	88.87	0.00

[a]Estimated by extrapolation.

[b]Includes from one to three pregnancies in which, because of early death of all embryos, there were no corpora lutea. In these cases the number of ovulated eggs was taken as 8.99, which is the mean number of corpora lutea per pregnancy for all pregnancies in which the corpora lutea were counted.

[c]There were no corpora lutea in any of these pregnancies. The number of ovulated eggs was estimated in the way described in footnote "b."

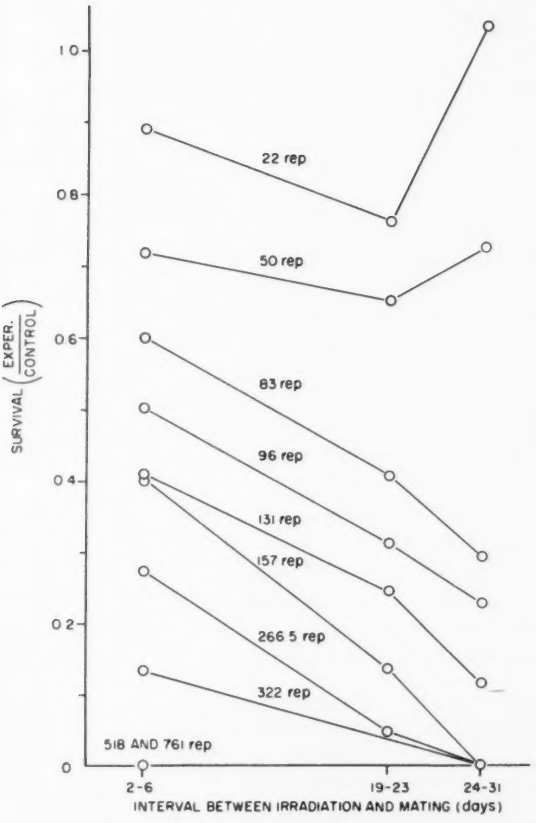

FIGURE 3. Dominant lethality in offspring of male mice mated at various times after exposure to various doses of radiation from nuclear detonation. Data from last columns of tables 1, 3 and 4 plotted as ratio of survival in the controls.

in the last mating period. The apparent inconsistency between the effects of these two doses and the effects of all other doses is presumably fully accounted for by the fact that, as was shown by further mating tests, the males exposed to these two doses showed no period of temporary sterility, while the males exposed to higher doses did. Furthermore, the interval between irradiation and the last mating period used for the obtaining of dominant lethal effects is long enough for a complete spermatogenic cycle to have occurred. It may be confidently assumed, therefore, that the spermatozoa used in the latest matings of the males exposed to the two lowest doses included cells that were spermatogonia at the time of irradiation, and that, as would be expected from X-ray experiments, these were virtually free of dominant lethal chromosomal changes. (The reason why spermatozoa irradiated as spermatogonia are free of such chromosomal damage is pre-

sumably because this type of damage occurring in spermatogonia would act as a cell lethal during spermatogenesis, leaving the production of spermatozoa solely to those spermatogonia which have escaped such damage.) It may be concluded that, for the irradiation of postspermatogonial cells, the results of the detonation experiment are consistent in showing an increase in dominant lethality with time of mating after irradiation.

Cyclotron data on the effect of time of mating are not as extensive. Table 5 gives the cyclotron results and also, for comparison, the results from the detonation for the same time intervals, and for similar levels of effect. It is apparent that for this effect, also, there is no significant difference between the results of the two experiments.

Summarizing the conclusions reached from the comparisons that have so far been made between the results of the detonation and cyclotron experiments, it can be said that, for comparable levels of effect, the two sets of results apparently do not differ either with regard to distribution of deaths according to age of embryos or with regard to increase in dominant lethality in later matings.

We can now turn to an examination of the results to see whether the quantitative response to neutron dose was different in the cyclotron and detonation experiments. This was considered the most important feature of the investigation when it was planned, and it was hoped that the physical dosimetry necessary for the comparison would, in spite of the difficulties of neutron measurement, and the added problems of instrumentation under

TABLE 5

COMPARISON OF EFFECT OF TIME OF MATING ON DOMINANT LETHALITY IN CYCLOTRON AND DETONATION EXPERIMENTS

	Total dose[a] (rep)	Matings made 2–6 days after irradiation	Matings made 19–28 days after irradiation		
		"Survivors" (data from tables 1 and 2) (per cent)	Number of pregnancies	Number of corpora lutea	"Survivors" (per cent)
Cyclotron	82	44	11	106[b]	25
	99	40	9	83[c]	10
	116	36	2	20	15
	141 and 160	25	2	20	10
Detonation	83	51	22	201[b]	26
	96	43	24	217[c]	24
	131	35	21	187[c]	18
	157	34	18	161[c]	9

[a]Gamma radiation component may be different in the two experiments.

[b,c]Includes respectively 1–2 and 3–5 pregnancies in which, because of early death of all embryos, there were no corpora lutea. In these cases the number of ovulated eggs was taken as the mean number of corpora lutea per pregnancy for all pregnancies, within that experiment, in which the corpora lutea were counted.

the field conditions, turn out to be of an adequate degree of accuracy. Since no methods were available for measuring neutron dose directly, it was planned to measure the total (neutron plus gamma radiation) dose inside each lead hemisphere with "tissue-equivalent" ion chambers and, by subtracting a separate measurement of the gamma radiation component, obtain an estimate of the neutron dose. The organization that was to have undertaken the ion chamber measurements was given other duties. Fortunately, Dr. C. W. Sheppard was able, at short notice, to design and have constructed a number of "tissue-equivalent" chambers suitable for measuring total dose over most of the range of doses used in the present study. Details of the method used are given by Sheppard and Darden (1954). The readings obtained inside the lead hemispheres at the various stations are shown in table 1, column 2. Sheppard and Darden discuss various possible errors in these measurements and conclude: "Taking all these complicating effects into account, we feel that it is highly unlikely that the ion chambers have overestimated the dose. They may conceivably have underestimated it but not by more than a factor of 2. It is not impossible that they were more accurate than we have supposed." Thus, the true total doses may have been higher than those recorded by the ion chambers, because "there is some evidence that... ion collection is impaired by the glutting effect of the high momentary ionization density." Actually, although this is not mentioned by Sheppard and Darden, this evidence came from a detonation other than the one under consideration here. For the detonation from which the present biological results were obtained, there are two independent lines of evidence, one physical, the other biological, that suggest that ion collection may have been essentially complete. The physical evidence comes from determinations of the flux of neutrons in the portion of the energy spectrum measured by sulfur threshold detectors. The curves obtained by plotting a linear transformation of these determinations and of the ion chamber readings against distance from the point of detonation are essentially parallel. The biological evidence was obtained in the following way. By comparing the dominant lethal results from the detonation with the cyclotron results, a dose estimate for each hemisphere was obtained in terms of the cyclotron dose in rep that would have produced the same biological effect. A least squares fit of these biologically estimated doses was then made to an equation in which dose is transformed to an expression that may be assumed to be approximately linearly related to distance. This fitted curve should have the same *slope* as one based on the true rep doses for the detonation, regardless of the gamma contamination and the relative biological effectiveness of the neutrons, in either the cyclotron or the detonation, provided that, within each experiment, the RBE is the same for different doses and the gamma contamination is a constant proportion of the total dose. The slopes would be approximately the same even if the RBE's vary, provided, as seems more likely, the ratio of the RBE's in the two experiments is the same at different doses. The slope obtained using the biologically estimated doses is -0.000617 ± 0.000058. Fitting the ion chamber

readings in the same way gives a slope of -0.000611 ± 0.000025. Thus, the slope of the fitted ion chamber readings does not differ significantly from the slope of fitted doses estimated from the biological effect. It is clear that there is no significant drop in ion chamber readings at closer distances as would have been expected if ion collection had been impaired by a glutting effect. This provides evidence, additional to, and independent of, that obtained from physical considerations, that ion collection was essentially complete in the ion chambers used in the detonation experiment considered here.

Turning to the gamma component of the radiation, this is estimated from film dosimeter readings made by the Radiation Instruments Branch, Division of Biology and Medicine, Atomic Energy Commission. Unfortunately, since the films have been shown to have some sensitivity to neutrons, the film dosimeter readings are not accurate estimates of the gamma doses. At the present time it is possible to estimate the contribution to the film dosimeter readings of neutrons with energies lying within the ranges affecting the gold and sulfur threshold detectors also used inside the hemispheres. Subtracting this from the film dosimeter reading for each station gives the gamma radiation estimates shown in table 1, column 4. They are presumably considerably higher than the true gamma doses because no allowance has been made for the unknown, but possibly appreciable, contribution of neutrons of intermediate energies. Minimum estimates of the gamma radiation obtained by the Radiation Instruments Branch are shown in table 1, column 3. These are based on the attenuation through seven inches of lead of the dose of gamma radiation recorded outside each lead hemisphere. The values are clearly minimum estimates of the true gamma doses inside the hemispheres, because they do not include any of the gamma radiation made by neutrons in the lead or inside the hemispheres.

With the large uncertainty about the gamma component of the radiation, it might at first appear that little could be said about the relative biological effectiveness of neutrons from the detonation as compared with neutrons in the cyclotron experiment. However, it will be shown that when both sets of gamma radiation figures are used to calculate minimum and maximum estimates of the ratio of biological effectiveness, these estimates turn out to be not very far apart. Taking the maximum estimates of the gamma radiation will give a maximum estimate of the RBE of the detonation neutrons, for if the true proportion of gamma radiation for a given total dose was lower than estimated, then the neutron dose must have been higher and the biological effectiveness of the neutrons would be less than that estimated. Similarly, taking the minimum estimates of the gamma radiation will give a minimum estimate of the RBE of the detonation neutrons. There remains the statistical problem of extracting the best point estimate and the confidence interval of both the minimum and maximum values. For statistical treatment the biological effect is expressed as percentage survival, table 1, column 11, and table 2, column 8. It is assumed that the logarithm of survival is linearly related to dose. Both the cyclotron and detonation data

give good fits on this interpretation, as shown in figures 4 and 5. The relation of biological effect to dose can then be expressed in the following way:

Let:

u_c = Biological effect, cyclotron

u_d = " " detonation

R_c = Total dose (rep), cyclotron

R_d = " " " detonation

G = Gamma dose (r), detonation

E_c = RBE, cyclotron neutrons compared with gamma

E_d = " detonation neutrons compared with gamma

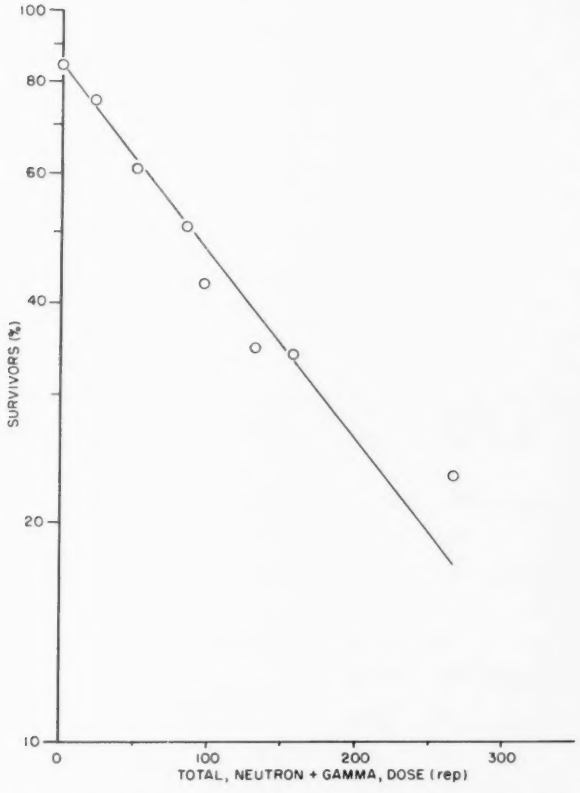

FIGURE 4. Dominant lethality in offspring of male mice mated from 2 to 6 days after exposure to nuclear detonation. Weighted least squares fit of logarithm of survival against dose. Experimental points from table 1, column 11.

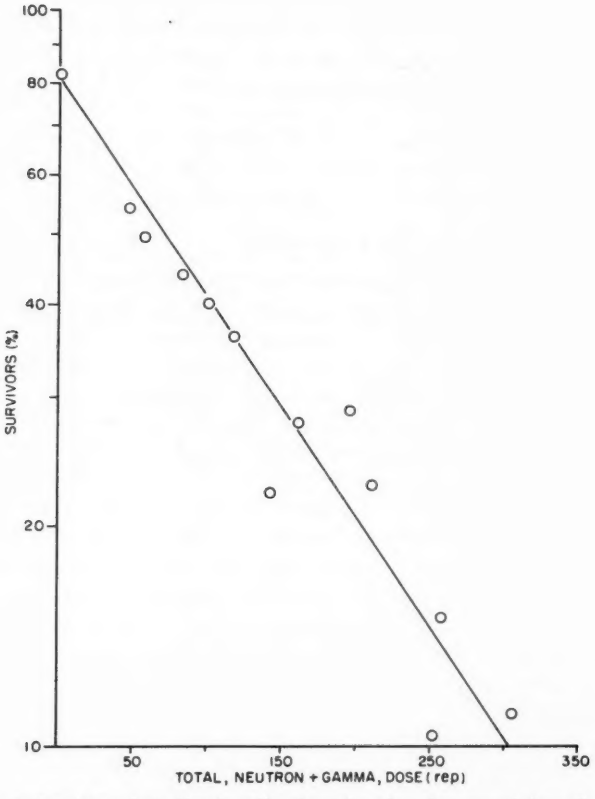

FIGURE 5. Dominant lethality in offspring of male mice mated from 2 to 6 days after exposure in cyclotron. Weighted least squares fit of logarithm of survival against dose. Experimental points from table 2, column 8.

Then, assuming additive effects of neutrons and gamma:

$$\log u_c = \alpha_c + \beta[0.9\, R_c\, E_c + 0.1\, R_c],$$

since 10 per cent of the total dose in the cyclotron was found to be gamma radiation, and

$$\log u_d = \alpha_d + \beta[(R_d - G)\, E_d + G].$$

These equations can be rewritten as:

$$y = \alpha_c + \beta x_1$$

$$z = \alpha_d + \beta' x_2 + \beta x_3$$

where:

$$y = \log u_c \qquad \beta' = \beta E_d$$

$$x_1 = 0.9\, R_c\, E_c + 0.1\, R_c \qquad x_2 = R_d - G$$

$$z = \log u_d \qquad x_3 = G$$

By the method of weighted least squares, the two equations were fitted simultaneously to the values given in tables 1 and 2, this quantitative comparison being restricted to the results of early matings which, as has already been pointed out, are likely to be less variable than the results of later matings. Data from the three stations closest to the detonation were excluded. Two separate fits were made, one using the minimum and the other the maximum gamma dose estimates. E_c was taken as 8.0. This is the best estimate available, but it is based on X-rays rather than gamma rays. It is the figure obtained by comparing the cyclotron results with data from an 800 r experiment with 250 kvp X-rays. An estimate of E_c determined from a gamma radiation experiment might turn out to be slightly higher, and E_c might vary somewhat with dose. However, as will be shown in another statistical treatment, even considerable error in the estimate of E_c would have little effect on the estimate of E_d/E_c. Minimum and maximum estimates of E_d were obtained from the ratio of the estimates of β' and β for each of the two fits. The corresponding minimum and maximum estimates of E_d/E_c, together with their 95 per cent confidence limits, are given in table 6. It should be pointed out that these estimates are based on the conclusion, discussed earlier, that ion collection was complete in the ion chambers used in this detonation experiment. If this was not so, then both minimum and maximum estimates would be lower if true total dose values could be used in place of the ion chamber readings. Thus, the present maximum estimate of E_d/E_c can be taken as a maximum with more assurance than the minimum estimate can be regarded as a minimum.

An alternative statistical treatment will show more clearly how the estimates of E_d/E_c are not much affected by error in the estimate of E_c. In this treatment it is assumed that the proportion of gamma radiation in the total dose is the same in each of the hemispheres used. Then, using the same symbols as before:

$$\log u_c = \alpha_c + \beta (0.9\, R_c\, E_c + 0.1\, R_c),$$

$$\log u_d = \alpha_d + \beta [(1 - p)\, R_d\, E_d + p\, R_d],$$

where p = the proportion of gamma radiation in R_d. These equations can be rewritten as:

TABLE 6

MINIMUM AND MAXIMUM ESTIMATES OF E_d/E_c, THE BIOLOGICAL EFFECTIVENESS OF DETONATION NEUTRONS RELATIVE TO CYCLOTRON NEUTRONS IN INDUCING DOMINANT LETHALS IN MICE

Estimate	$\frac{E_d}{E_c}$	
	Point estimate	95 per cent confidence limits
Minimum	0.80	0.67 and 0.96
Maximum	1.18	0.91 and 1.55

$$y = \alpha_c + \beta_c R_c$$

$$z = \alpha_d + \beta_d R_d$$

where: $y = \log u_c$ $\qquad z = \log u_d$

$$\beta_c = \beta(0.9 E_c + 0.1) \qquad \beta_d = \beta[(1 - p) E_d + p]$$

A minimum estimate of p was obtained by a least squares fit of the minimum gamma dose estimates against the total dose estimates. A maximum estimate of p was obtained in the same way from the maximum gamma dose estimates. These minimum and maximum values are 0.0216 and 0.376, respectively. Using these values of p, the equations, fitted by the method of weighted least squares, give minimum and maximum values of β_c and β_d. Minimum and maximum estimates of E_d/E_c can be obtained from the relation:

$$\frac{E_d}{E_c} = \frac{0.9\,\beta_d}{(1-p)\,\beta_c} + \frac{1}{E_c}\left[\frac{0.1\,\beta_d - p\,\beta_c}{(1-p)\,\beta_c}\right],$$

which is derived by equating the two estimates of β given by β_c and β_d. For reasonable values of E_c, the second term in this expression is small compared with the first term. Therefore, even a large error in the estimate of E_c will have little effect on the estimate of E_d/E_c. When E_c is taken as 8.0, the minimum and maximum estimates of E_d/E_c are 0.80 and 1.18, respectively. It has been stated earlier that the true value of E_c may be somewhat higher than 8.0, but even if E_c is taken as twice as large, the minimum and maximum estimates of E_d/E_c are changed only slightly, namely, to 0.79 and 1.21, respectively. It may be noted that the estimates obtained when E_c is taken as 8.0 are in agreement with those from the earlier statistical treatment.

From both statistical treatments, it is clear that, under the conditions of this test, the biological effectiveness of the detonation neutrons is not significantly different from that of the neutrons in the cyclotron experiment.

It is perhaps of some interest to assume that the ratio of biological effectiveness is, in fact, unity, to assume that the ion chambers correctly estimated the total dose, and then calculate what percentage of the total dose has to be assigned to gamma contamination to give the observed biological results. Fitting the results to these assumptions gives a point estimate of the gamma component of 25 per cent, with 95 per cent confidence limits of 7.4 and 40 per cent.

In conclusion, it may be said that, so far as dominant lethals in mice are concerned, there is no evidence of qualitatively different effects of neutrons in the detonation and cyclotron experiments. There is also no significant difference in the quantitative results of the two experiments. With regard to the practical problem of human hazards, it is of first importance to consider an upper probability limit of the biological effect. Fortunately, as has been pointed out, the estimated maximum ratio of biological effectiveness can be regarded as a maximum with considerable assurance, because if corrections could be made for an almost certain error in the gamma dose

estimates and a possible error in the total dose estimates, both corrections would lower the estimated maximum ratio of biological effectiveness. Since the upper 95 per cent confidence limit of this estimated maximum ratio is 1.55, it seems safe to assume, as a starting point in the extrapolation of mouse data to man, that it is unlikely that the hazard, from the intensity and the spectrum of energies, of the neutrons encountered in the detonation experiment is more than one and a half times that of the neutrons in the cyclotron experiment. It should be emphasized that this is an upper limit: the hazard may actually be no greater.

It must be kept in mind that this comparison between the effects of laboratory and detonation neutrons is based on physical methods of dose estimation which, although they appear to be satisfactory, have not as yet been subjected to sufficiently rigorous tests. It is hoped that it will soon be possible to improve the laboratory side of the comparison by using a neutron facility designed to permit simpler and more precise radiobiological experiments.

SUMMARY

Dominant lethality in the offspring of male mice exposed in lead hemispheres to neutron radiation from a nuclear detonation was determined for ten different doses by mating the males to unexposed females, dissecting the females at a late stage in pregnancy and recording the number of living and dead embryos, resorption sites and corpora lutea. Data from early matings (2 to 6 days after irradiation) and late matings (19 to 31 days after irradiation) were tabulated separately.

Comparison of the results with those from a similar experiment with fast neutrons from a cyclotron shows:

1. For comparable levels of total effect, the two sets of results do not differ significantly in the distribution of deaths according to age of embryos.

2. The increase in dominant lethality observed when the offspring of late matings are compared with those of early matings is similar in the two experiments.

3. The biological effectiveness of detonation neutrons relative to cyclotron neutrons lies between 0.80 and 1.18, the minimum and maximum estimates obtained when allowance is made for uncertainty in the physical measurements of the gamma radiation contamination in the detonation experiment. (Taking the biological effectiveness of cyclotron neutrons relative to X-rays as 8.0, the corresponding minimum and maximum estimates of the biological effectiveness of detonation neutrons relative to X-rays are 6.4 and 9.4, respectively.)

It may be concluded that, although there is a marked difference in intensity, and presumably some difference in energy spectrum, between the detonation and cyclotron neutrons, the present data show no significant difference in the effectiveness of these neutrons in inducing dominant lethality in mice.

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THE PRODUCTION OF TRANSLOCATIONS IN *DROSOPHILA VIRILIS* BY FAST NEUTRONS FROM A NUCLEAR DETONATION[1,2]

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The Division of Biology and Medicine of the Atomic Energy Commission provided us with an opportunity to measure genetic damage induced by fast neutrons from two nuclear detonations. Except for the radioactive isotopes, which were not studied separately, the ionizing radiations are produced within a fraction of a second. Conger (1954) has described the neutron stations which were spaced at different distances from the center of the detonation. *Drosophila virilis* placed in these stations were screened from gamma and other ionizing radiation to a large degree by the seven inch thick lead walls, and protected against excessive heat by a temperature control system.

Drosophila virilis males of the Texmelucan strain, 6 to 9 days old at the time of irradiation, were mated individually within twenty-four hours to three females marked by the mutants *broken* (*b*, chromosome 2), *tiny bristle*, *gapped* (*tb gp*, chromosome 3), *cardinal* (*cd*, chromosome 4) and *peach* (*pe*, chromosome 5). After three days the males were discarded so that sperm mature at the time of irradiation were tested. The F_1 males were backcrossed to females carrying the five mutant marker genes and translocations were scored genetically in the progeny from the backcross. This method was used by Baker (1949), Baker and Edington (1952) and Haas *et al.* (1954), and its advantages and disadvantages as a measure of genetic damage from irradiation are discussed in those papers. The translocation test for fast neutron damage to the genetic system has the advantage that it may be used to distinguish between the "one hit" and "multiple hit" phenomena of radiation effects.

RESULTS

The rates of production of translocations at the biological test stations at different distances from the nuclear detonation are given in table 1 and plotted on figure 1. The estimated dose in rep on each station used in these two tests, A and B, was calculated by Dr. C. W. Sheppard. Table 1 gives the number of translocations of different types, T_2 designates a translocation involving two chromosomes and T_{2+2} denotes one with two separate translocations in one individual. In table 1 are also listed the number of sperm tested, the estimated dosage for each station, the number of sperm

[1]Work supported by Contract AT-(40-1)-1323 with the Atomic Energy Commission.

[2]This paper, although not a part of the Symposium, is related in that it contains material obtained at the same nuclear tests as the material for the Symposium papers.

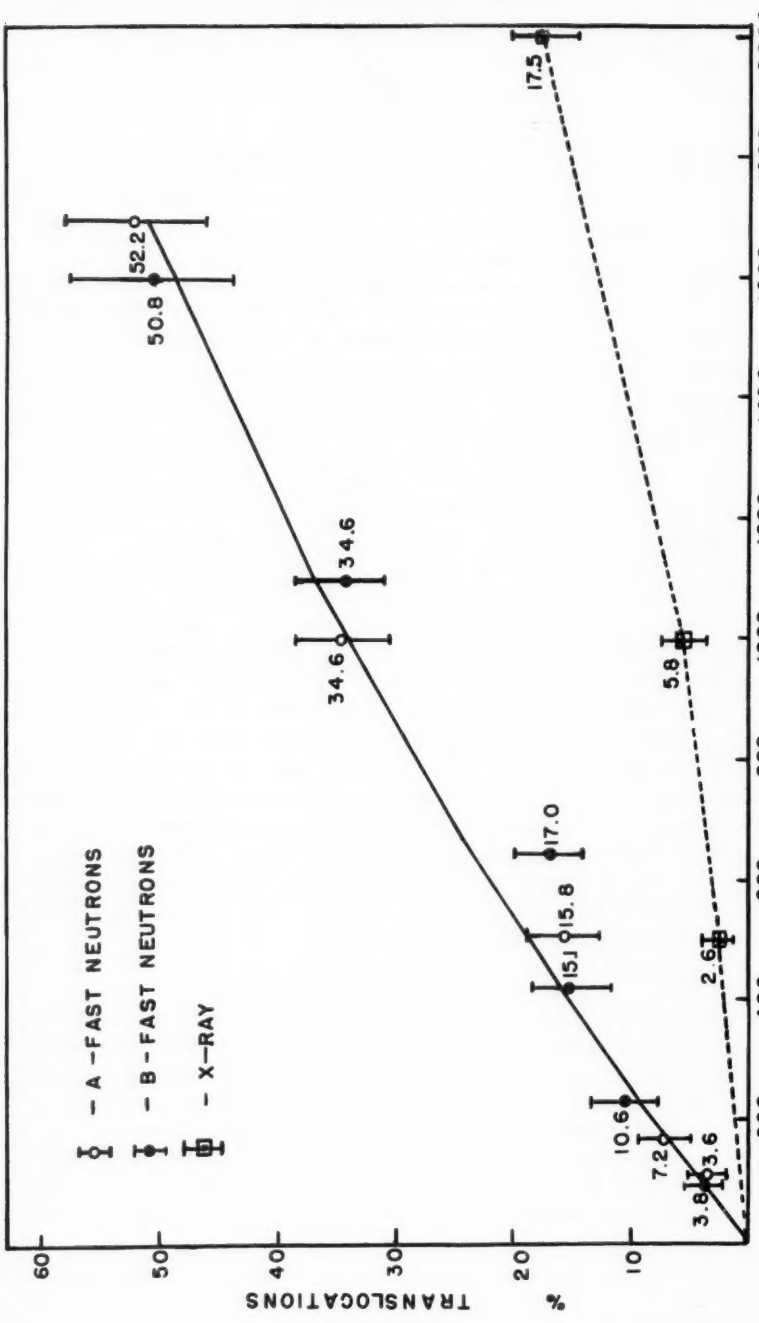

FIGURE 1. Dosage—Translocation Rate Curves.

TABLE 1

FAST NEUTRON DAMAGE TO GENETIC SYSTEMS: TRANSLOCATIONS

Test	Estimated rep	Types of Translocations						All separate translocations	Number normal sperm	Total sperms tested	Per cent separate translocations ± SD
		T_2	T_3	T_{2+2}	T_4	T_{2+3}	T_5				
A	3500	Genetic sterility—motile sperm in P_3 ♀ and eggs but no offspring									
A	1700	85	23	11	12	1	1	145	122	255	56.9 ± 3.1
A	1000	134	28	14	7	2	1	202	352	538	37.5 ± 2.1
A	510	76	6	0	0	0	0	82	436	518	15.8 ± 1.6
A	166	31	4	0	0	1	0	37	464	500	7.4 ± 1.2
A	106	17	1	0	0	0	0	18	485	503	3.6 ± 0.8
B	1600	63	25	8	4	0	0	108	97	197	54.8 ± 3.5
B	1100	148	26	7	7	2	0	199	359	549	36.2 ± 2.0
B	640	86	14	1	1	0	0	103	499	601	17.1 ± 1.5
B	420	71	4	1	0	0	0	77	427	503	15.3 ± 1.6
B	230	44	9	1	1	0	0	56	453	518	10.8 ± 1.4
B	94	18	1	0	0	0	0	19	481	500	3.8 ± 0.9
X-ray	500 r	14	0	0	0	0	0	14	555	569	2.6 ± 0.7
X-ray	1000 r	49	0	0	0	0	0	49	809	858	5.8 ± 0.8
X-ray	2000 r	109	10	2	0	0	0	123	582	703	17.5 ± 1.4

without a translocation, and the number in each translocation class. The column "All Separate Translocations" is obtained by counting T_{2+2} and T_{2+3} as two translocations. This column is used to calculate the per cents of separate translocations with their standard deviation as given in the last column.

These translocation rates are plotted in figure 1 against the estimated rep calculated for each biological test station. Previous experiments reviewed by Lea (1946), Catcheside (1948), Fano, Caspari, and Demerec (1950) and Mulier (1954) show that chromosome breakage and chromosome rearrangements are "one hit" phenomena in the sense that one proton track from the fast neutron bombardment ordinarily causes the several chromosome breakages involved in a translocation. For this reason it is often stated that the rate of production of translocations is directly proportional to the dosage of fast neutrons. If this is true the curve would approximate a straight line with low doses. The curve would not be a straight line at higher doses because of saturation; therefore, a theoretical curve taking into account this effect is shown in figure 1. In our data the four lower doses of neutrons should seldom include cases where two effective tracks occurred in the same cell. An average of 4.1% translocations per 100 rep obtained from these four tests was used to calculate the points for the curve. This was done on the basis that each additional 100 rep induced translocations in 4.1 per cent of the sperm as yet free of translocations. These would be detectable as new translocations, whereas those that occurred in the sperm with other translocations might not be detected as separate events.

Nine of the eleven experimental points fall on the curve based on the average rate for the four lower doses. Two stations with medium doses gave translocation frequencies so low that they fall significantly below this line but there is good agreement for the four higher doses. Therefore these data support the theory that the number of translocations produced is directly proportional to dosage. This conclusion implies that only one track from a proton displaced by the fast neutron bombardment is necessary to produce all the chromosome breaks involved in any translocation.

Although the X-ray tests plotted in figure 1 were delivered at 1818 r per minute, the rate of irradiation was slower than that of the gamma contamination from the nuclear detonation. Even though the gamma contamination might have contributed an appreciable amount to the measured dose (Dr. Sheppard estimated that it was under one-third) it could not have modified the results appreciably except at the higher doses where it may have produced a small percentage of the translocations. The neutron and X-ray translocation rate curves do not resemble each other since neutrons produce translocations by "single hits" and X-rays involve "multiple hits."

The fertility of the P_1 males was high, ranging from 83 to 99 per cent, with one exception. However, heavy irradiation reduced the number of offspring. Also, about one-third of the F_1 males coming from sperm subjected to 1000 rep or over were sterile; however, those from P_1 males irradiated

with 510 rep were 85 per cent fertile. The sterility of the P_1 males irradiated with 3500 rep was due primarily to genetic damage. These males inseminated females, the females retained motile sperm for a number of days, and most of a sample of eggs which were examined had been fertilized. A few larvae developed and one or two adult flies emerged from several thousand eggs. The death of the F_1 zygotes between the fertilization of the egg and emergence of the adult probably resulted from the presence of lethal chromosome abnormalities (e. g. dicentrics, rings, etc.) in almost all sperm. This result is in agreement with those obtained by Baker and Von Halle (1954) who used egg hatch to measure dominant lethals produced by neutrons from these nuclear detonations.

The complex translocations listed in table 1 give us information on the joining of chromosome ends broken by radiation. Lea and Catcheside (1945), Haldane and Lea (1947) and Catcheside (1948) calculated the relative frequency of different types of rearrangements possible with different numbers of breaks in the chromosomes. If broken chromosome ends unite at random, twice as many T_4 as T_{2+2} type translocations would be expected. This is not the case in Tradescantia, as they point out. These data and those of Baker (1949) and Stone *et al.* (1954) indicate that the joining of broken chromosome ends is not at random. In the present study 43 T_{2+2} and 32 T_4 types were found although the ratio expected from random attachment would be 1:2. There is less than one chance in a hundred that these class frequencies could result from random combination of broken chromosome ends.

DISCUSSION AND CONCLUSIONS

Fast neutrons are much more effective than X-rays in producing genetic damage measured as translocations. This difference is more marked at lower doses. A rough equivalence in damage exists for 100 rep and 750 r, 500 rep and 2000 r and 1300 rep and 4000 r (using the last value from Baker, 1949). The direct proportionality between fast neutron dosage and translocation frequency indicates that small doses of neutrons are relatively more dangerous to genetic systems than small doses of X-rays. Our estimates of equivalent damage showing the difference in effectiveness of neutrons and X-rays agree with the report of Baker and Von Halle (1954) using dominant lethals in Drosophila. Ehrenberg, Gustafsson and Nybom (1952) and Ehrenberg and Nybom (1952) estimate that neutrons are 10 to 20 times as effective as X-rays in producing chromosome rearrangements and even more effective in producing mutations in barley. These workers find a direct proportionality to dosage as does Giles and coworkers (1950, 1952, 1953). Dose fractionation with neutrons makes no difference in the rate of production of rearrangements in Tradescantia although it does with X-rays (Giles, *et al.*, 1953). Conger (1954), Kirby-Smith and Swanson (1954), Baker and Von Halle (1954) give data on fast neutrons from a cyclotron, uranium fission and these nuclear detonations. They conclude that radiation damage, including the production of translocations, is directly proportional to dosage. Our results agree with this conclusion.

The complex translocation types, T_4 and T_{2+2}, should occur in a two to one ratio if attachment of broken chromosome ends is at random. Table 1 shows that this is not the case, but that there is a decided bias in favor of two independent translocations. In these fast neutron studies the excessive 2 by 2 attachments cannot be the result of a failure for all breaks to be present at one time. Similar results have been obtained with X-rays (Stone, *et al.*, 1954). This suggests that proximity favors union in Drosophila as postulated for Tradescantia by Lea and Catcheside (1942). Chemical selectivity in the union of broken chromosome ends may also be an important factor.

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SURVIVAL AND MUTATION IN NEUROSPORA EXPOSED AT NUCLEAR DETONATIONS[1,2]

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In the experiments to be described here, the conidia of a fungus, *Neurospora crassa*, were exposed to radiation from nuclear devices. Biological information of three types was obtained: first, the survival of conidia under conditions of forced heterokaryosis, where the viability of two or more genetically different nuclei per cell is a prerequisite of survival; second, the survival on supplemented medium, where survivors need contain but a single viable nucleus per cell; and third, the frequency of nuclei carrying recessive lethal mutations. These effects were measured after exposure at several stations, both inside and outside the seven-inch-thick lead neutron hemispheres in detonations A and B. It must be emphasized at the beginning that those conclusions which depend on exact knowledge of the radiation dose must not be taken too seriously, because the material is sufficiently resistant to radiation to necessitate its being placed where no physical measurement of total rep could be made by available methods, not a surprising eventuality when it is realized that dose rates of the order of billions of rep per second were involved. The magnitude of the error involved in estimates by extrapolation is unknown. However, the findings justify several conclusions which are independent of absolute dose measurements; namely, that the radiation from these devices kills the conidia by damaging the cell nuclei, and not through damage to other cell constituents; that a slow neutron component of the radiation was detected biologically, and that exposure under anoxic conditions (nitrogen atmosphere) had no protective effect. On the whole, the results give no reason to believe there is anything unique about the biological effects of radiation from the devices; anything which could not be duplicated by mixed radiation from laboratory sources. In order to understand the results we must first discuss the special properties of the material which facilitate these conclusions.

HETEROKARYOSIS AND THE ANALYSIS OF RADIATION EFFECTS

The stock used in the present experiments contains two genetically different types of nuclei. This condition is known as heterokaryosis. One of the nuclear components carries a mutation leading to a requirement for arginine, whereas the other component carries mutations leading to a requirement for methionine and to a drastic morphological modification known

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[2]This paper, although not a part of the Symposium, is related in that it contains material obtained at the same nuclear tests as the material for the Symposium papers.

as amycelial. In a homokaryotic condition; that is, where only one of the two nuclear types is present, each of these mutations is fully expressed phenotypically: an arginine culture will not grow unless arginine is provided in the medium, and a methionine-amycelial culture will grow only if methionine is present, and will show the special morphology caused by the amycelial mutation. In the heterokaryon, however, none of the mutations are phenotypically expressed. The arginine nuclei contain wild-type genes capable of bringing about the normal synthesis of methionine, and of offsetting the effects of the amycelial mutation; the methionine-amycelial nuclei contain wild-type genes sufficient for the normal synthesis of arginine. Thus, when both nuclear types are together in the same cytoplasm, a complete set of genes is active, and phenotypically normal growth occurs without the addition of the amino acids to the medium. Such complementary action appears to be characteristic of nuclei carrying different biochemical mutants (Beadle and Coonradt, 1944).

Neurospora normally grows as a mycelium in which many nuclei coexist in a common cytoplasm. When conidia are formed at the terminal branches of the mycelium, one or more nuclei are included in each cell, a majority of the cells being multinucleate. Distributions of numbers of nuclei per conidium have been published several times (Atwood and Norman, 1949; Norman, 1950; Huebschman, 1952; Prout *et al.*, 1953). In the present case, the average number of nuclei per cell is about three. When conidia are formed by the heterokaryotic culture, their nuclear constitution is determined by a primarily random assortment of nuclei of each type into the multinucleate cells, although non-random processes are superimposed, often leading to a significant excess of homokaryotic cells (Prout *et al.*, 1953). Thus, three types of conidia are formed which differ in nuclear constitution; first, there are conidia which are homokaryotic for arginine; second, those which are homokaryotic for methionine-amycelial; third, those which are heterokaryotic; that is to say, those which contain at least one nucleus of each type. The relative frequencies of the three types in the total population of cells can be determined from the differences in viable count on different media. The minimal medium, which contains neither arginine nor methionine, will support the growth of the heterokaryotic cells only. Medium containing methionine will support the growth of the methionine-amycelial homokaryons in addition to the heterokaryotic cells. Medium containing both arginine and methionine will support the growth of all the cells in the population.

It now becomes clear how the distinction can be made between the survival of whole cells and the survival of cell nuclei. If an agent kills the cells by independently inactivating the nuclei, then the proportion of heterokaryotic cells among the survivors will very rapidly decrease as the surviving fraction of cells decreases. On the other hand, if an agent kills the cells by a process in which the nuclei are not primarily inactivated—in other words, if the lethal effects on the whole cells predominate and are independent of those on the nuclei—then the fraction of heterokaryotic cells among the survivors will tend to remain constant despite the decrease

in cell survival. By the method of determining the heterokaryotic fraction in treated and control cells, inactivation of the nuclei has been shown to be the cause of cell death by X irradiation, whereas killing of the cells by heat, for instance, has been shown to be non-nuclear (Atwood, unpublished). Where the lethal effect is solely on the nuclei, the expected survival, S_{min}, on minimal medium, on which only the heterokaryotic cells can grow, is related, as a rough approximation, to the survival, S, on supplemented medium by the expression:

$$S_{min} = \frac{1}{2} S^2. \qquad (1)$$

This expression holds rather well for conidia treated with X-rays or with cyclotron neutrons, but it has not yet been determined by laboratory experiments whether it holds for all ionizing radiations. The relation does not, of course, hold where S is close to unity, but otherwise it is correct within a factor of two or three, and greater deviations may be taken to indicate that some inviability has been caused by agents having an action different from that of X-rays.

RECESSIVE LETHAL MUTATIONS

Recessive lethal mutations are genetic changes in the nuclei which are viable if other nuclei are present bearing the corresponding normal genes. In this respect they resemble the biochemical marker mutations already present, but most of them differ from biochemical mutants in being inviable in the homokaryotic condition regardless of the composition of the external medium (Atwood and Mukai, 1953). In order to detect recessive lethal mutations (Atwood, 1949), the irradiated cells are first spread on minimal medium. All cells which survive and grow on this medium must be heterokaryotic, since no growth factors are supplied. Each of these survivors is isolated and allowed to produce its own conidia. These conidia are of the three types which have previously been mentioned; namely, two homokaryotic types, requiring arginine and methionine respectively, and the heterokaryotic type. Now, if any additional mutations have been induced, other than the arginine and methionine mutations which are already present in the nuclear components, the conidia which become homokaryotic for these new mutations will be unable to grow even though they are supplied with the growth factor which they ordinarily require (arginine or methionine). In other words, the method of detecting recessive lethal mutations is to allow the treated nuclei, which are viable in heterokaryotic cells, to reproduce extensively, reassort themselves, and give rise to homokaryotic cells, and then to determine whether these homokaryotic cells are viable. The presence of the amycelial mutations in the methionine nuclei greatly facilitates this process, since it is only necessary to determine whether the amycelial homokaryons in the conidial population from an isolate are viable in order to tell whether a recessive lethal mutation has been induced in the methionine-amycelial nucleus. This may be seen at a glance when the mixed conidia

of an isolate have been incubated on methionine medium. If no mutation has been induced, both the normal heterokaryotic and the amycelial colonies will be present. However, if a mutation has been induced in the methionine-amycelial nucleus of the original conidium, then only colonies of normal morphology, which are heterokaryotic, will appear. Once obtained, the mutants may be kept as heterokaryons and analyzed according to various other criteria (Atwood, 1950; Atwood and Mukai, 1953, 1954).

MATERIALS AND METHODS

Conidia from week-old cultures of the heterokaryon between arginine (29997A), and methionine-amycelial (4894-422A) were transferred as semidry clumps to the test containers. The containers were of thin lusteroid, 13 × 65 mm, or of 2S aluminum, 13 × 75 mm with ca. 1 mm wall thickness. The capped ends were dipped in paraffin of 55 °C melting point and the containers were sealed in groups of two to four in thin-walled plastic bags. Lusteroid containers were placed in the neutron hemispheres, and the aluminum containers outside the hemispheres in iron pipes. These pipes were of $\frac{5}{8}$ inch inside diameter, 36-inch length, and were driven into the ground to leave only 8 inches protruding. The lumen was blocked by a cross pin at ground level and the top 8 inches of pipe received two aluminum containers enclosed in a narrow cylindrical plastic bag. A cotter pin across the top of the pipe prevented the contents from being ejected. A special aluminum container was devised for exposing material in an atmosphere of nitrogen. It is similar to the standard container, but has a threaded cap at one end and a narrow neck at the other. After loading, the cap was sealed with glyptal and the chamber flushed ten times through the neck with nitrogen. The chamber was sealed under nitrogen at 5 lb per sq in by pinching the neck with round nosed pliers. The seal was confirmed by holding the containers under water, and pressure in the containers was reconfirmed at the time of opening.

The times of placement and recovery are not critical for experiments with semidry Neurospora conidia. After recovery, the material was returned to Oak Ridge, the caps removed, and the conidia brought into aqueous suspension. The suspensions were appropriately diluted and poured into petri plates with sorbose agar medium. When it was necessary to know the relative numbers of cells in different suspensions, the turbidity of undiluted suspensions was determined in a Coleman nephelometer. Every experiment included one or more controls which were prepared at the same time, placed in the same types of containers, taken to the test site and carried with the experimental material at all times except in the actual exposure. The assignment of significance levels to the survival data is impracticable in these experiments, mainly because of erratic fluctuations in the control viable counts. There is little doubt that this variability was produced by non-radiomimetic factors, since the heterokaryotic fractions remained essentially constant despite variations of as much as 20 per cent in viable count relative to turbidity.

There were several variations in composition of the media from which conidia were harvested, and on which they were plated. The former will be referred to as "media of origin" and the latter as "plating media." The purpose of using different plating media has already been explained. The purpose of using different media of origin was to vary the elemental composition of the conidia in such a way as to change the capture cross section for slow neutrons. In some media N^{15} as nitrate, was incorporated to the extent of 65 atoms per cent. This isotope has a capture cross section about 10^{-3} that of N^{14}, and should therefore decrease the sensitivity to thermal neutrons. In some media, B^{10} was added to sensitize the material to thermal neutrons. In all media the agar was thoroughly washed in an attempt to avoid uncontrolled trace element contributions. The plating media contained the standard salt mixture of Neurospora minimal (Beadle and Tatum, 1945) without tartrate, and with 0.1 per cent sucrose and 1.0 per cent L-sorbose. Supplements of 25 μg of L-methionine or L-arginine per milliliter, or both, were added where indicated. The medium of origin for the detonation A experiments was standard Neurospora minimal with the trace element mixture and boron content varied where indicated. Boron additions were made as borate. For the detonation B experiments the nitrogen source was solely nitrate, as in the medium of Westergaard and Mitchell, 1947. The N^{15}-labeled nitrate, supplied by Eastman Kodak Company, was substituted where indicated.

SURVIVAL OF CONIDIA IN THE NEUTRON HEMISPHERES

Conidia produced on standard medium and enclosed in lusteroid containers were recovered from four positions at detonation A. Survival of cells plated on minimal methionine, arginine, and doubly supplemented medium is expressed in each case as the fraction of the control count on the corresponding plating medium, appropriate corrections having been applied for differences in nephelometer readings and dilutions. The proportions of heterokaryotic cells and of the two types of homokaryotic cells are obtained by subtracting the plate counts on minimal medium from the counts on each singly supplemented medium, then dividing each difference and the minimal count by the total. The results are shown in table 1.

The last column in table 1, the ratio of the observed to expected total counts, requires further explanation. The total viable cells may be estimated in two ways: by summing the minimal count and the two differences (expected count), or directly, by using the count on doubly supplemented medium (observed count). These results should agree within sampling error if the plating efficiencies on all media are equal. Since this is not always the case, it is desirable to confirm the agreement within a given experiment. It may be noted that the frequency of methionine-amycelial homokaryons can also be estimated in two ways: by difference, using minimal and methionine plates, or by differential counting of the amycelial colonies on the methionine plates. These methods agree well, but the difference of two sets of plates is the method of choice in irradiated material because of

TABLE 1

SURVIVAL ON THE FOUR DIFFERENT PLATING MEDIA, AND THE PROPORTIONS OF THE THREE CELL TYPES

Standard medium of origin. Inside neutron hemispheres, detonation A.

Estimated neutron dose (rep)	Estimated gamma dose (rep)	Surviving fractions on indicated plating medium				Proportions of the three cell types			Ratio of observed total count to expected total count
		Minimal	Methionine	Arginine	Methionine + arginine	Heterokaryotic	Homokaryotic		
							Methionine	Arginine	
7000	10,000	0.15	0.47	0.43	0.55	0.15	0.49	0.36	1.07
4000	4,000	0.39	0.68	0.68	0.80	0.29	0.39	0.32	0.99
3500	3,000	0.51	0.77	0.74	0.88	0.35	0.37	0.28	0.96
2500	1,900	0.49	0.70	0.73	0.80	0.36	0.34	0.30	0.98
Control						0.63	0.23	0.14	0.92

the confusing variety of morphological anomalies appearing at the high doses.

It is evident from table 1 that the effect on survival is mediated through the cell nuclei, as it is with X-rays, since the survival of heterokaryotic cells (on minimal plating medium) decreases more rapidly than that of the total, and the proportion of homokaryotic cells among the survivors increases with the dose (Atwood, 1952). Thus we infer that the heterokaryotic cells are, by lethal effects on the nuclei, transformed into homokaryons; and these, in turn, are killed by effects on the surviving nuclei, an inference which is amply supported by the remainder of the experiments to be presented.

THE SIGNIFICANCE OF THE HETEROKARYOTIC FRACTION

The complete presentation in table 1 serves to illustrate the elements of the experiment, but the essential meaning of the result may be seen from the relative survival on minimal and doubly supplemented plating media alone. Therefore, in the remaining experiments, other survival data will be omitted. The heterokaryotic fractions will bear close scrutiny, however, because they serve the valuable purpose of detecting non-radiomimetic effects. For instance, if the survival of a sample is too low to be consistent with others receiving the same dose, the heterokaryotic fraction is examined. If it is found to agree with those of the other samples, it may be inferred that the anomalous low survival is an artifact, such as might be caused by the handling of the material under field conditions.

In order to test simultaneously all the data in this way, a theoretical relation between the survival and the heterokaryotic fraction was derived. Where equation (1) is valid, it can be shown that the heterokaryotic fraction and the survival on minimal plating medium are related by the expression

$$S_{min} = 2r^2/c^2, \qquad (2)$$

where S_{min} is the survival on minimal plating medium, r is the heterokaryotic fraction of the total viable cells in the irradiated population, and c is the heterokaryotic fraction in the unirradiated control. The theoretical relation between S_{min} and r is plotted in fig. 1 for a fixed value of $c = 0.63$, the average heterokaryotic fraction in the controls. The points in fig. 1 are experimental, each point a different sample of conidia. They comprise all the data from the nuclear detonations, with X-ray data for comparison. The degree of deviation from the theoretical curve which should be regarded as anomalous is, in the present experiments, a matter of subjective judgement. Several factors are taken into account; e.g., the over-all variability, the results of previous experiments, and the fact that the approximations on which equations (1) and (2) are based are not good in the first decade of survival, where the agreement with theory is largely a fortuitous result of the initial distribution of nuclei in the conidia, and must therefore be determined empirically. On such considerations, deviations from theory by a factor of three or more, with survival below 0.1, were thought to be definitely

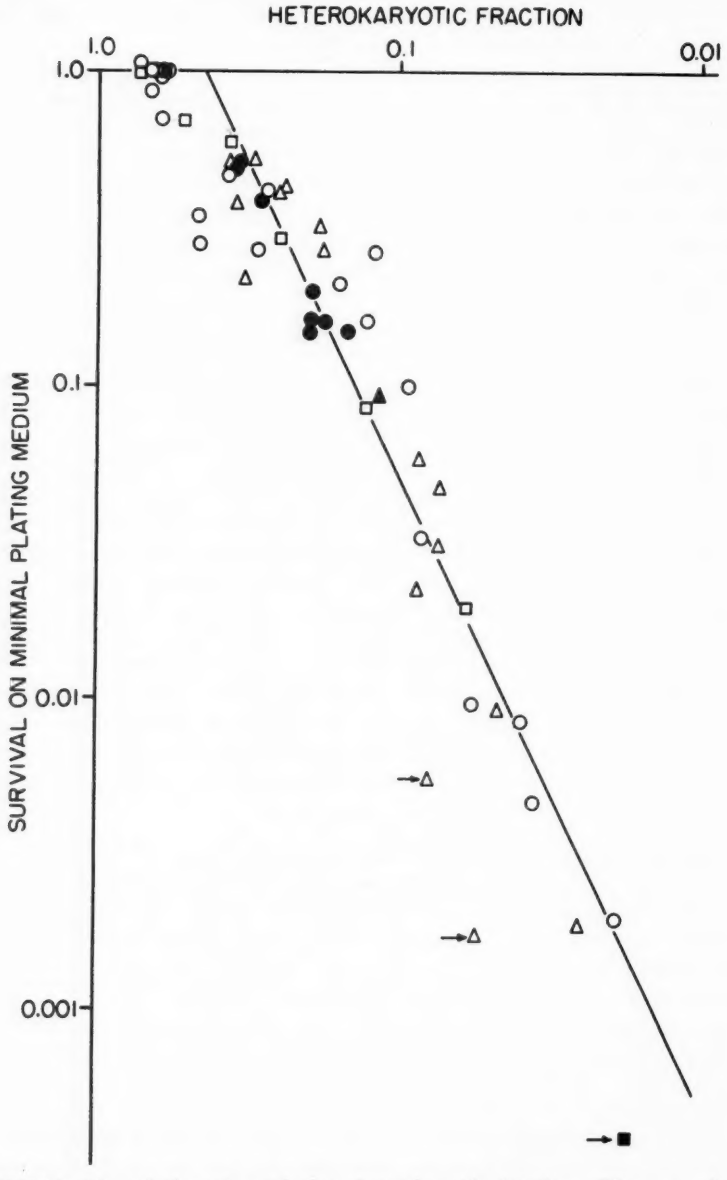

FIGURE 1. The relation of survival to heterokaryotic fraction. The curve is theoretical, from equation (2), with c = 0.63. The points are experimental: ● = detonation A, inside hemispheres; ○ = detonation B, inside hemispheres; △ = detonation B, outside of hemispheres; ▲ = exposed in N_2, detonation A; ■ = exposed in N_2, detonation B; □ = 250 kvp X-rays with 3 mm of Al filtration. The points indicated by arrow are considered to be anomalous.

anomalous. Three such cases, all from detonation B, were found: two exposed outside the hemispheres, and one exposed in nitrogen. In all three the survival is too low, indicating that they sustained some additional lethal effect not caused by radiation. The remaining data may be taken as an over-all confirmation of the hypothesis of nuclear inactivation.

EFFECTS OF BORON-10 AND NITROGEN-15 ENRICHMENT

It has been shown in Tradescantia that a large portion of the biological effect of thermal neutrons is caused by the n,α reaction of boron (Conger and Giles, 1950; Conger, 1953). The capture cross section of B^{10} (3800 barns) is such that the element becomes important in trace amounts.

Conidia from mycelia grown on four media of origin differing in boron content were recovered from a hemisphere after detonation A. These media were: trace element-free; boron-free; 1 ppm natural boron, and 1 ppm B^{10}. The natural boron contains 18.8 per cent B^{10}. The survival on minimal and supplemented media and the heterokaryotic fractions are given in table 2. The results are in good agreement with those from standard medium (containing 0.01 ppm of natural boron), and the trend toward lower survival in the boron-enriched material is quite inconclusive. Conidia exposed in detonation B provided a greater range of differences in slow neutron capture cross section than in the previous experiment. The media of origin were as follows:

N^{15} : Contains 65 atoms per cent N^{15}.

Normal: Boron free, with natural nitrogen.

$N^{15}B^{10}$: Contains 65 atoms per cent N^{15} and 16 ppm of B^{10}.

B^{10} : Contains 16 ppm of B^{10}.

Fig. 2 shows the survival on minimal and doubly supplemented plating media of cells from these media of origin. The control counts, corrected for nephelometer reading, differed among the different media of origin by as much as 20 per cent. Since the heterokaryotic fraction was found to be essentially the same in all controls, these differences are interpreted largely

TABLE 2

SURVIVAL OF CONIDIA HARVESTED FROM VARIOUS MEDIA AND PLATED ON MINIMAL AND SUPPLEMENTED MEDIA

Detonation A, estimated dose: neutron—7000 + gamma—10,000 rep. Exposure in neutron hemisphere

	Plating medium	Medium of origin			
		Trace element free	Boron free	1 ppm of natural boron	1 ppm of boron-10
Survival	Minimal	0.20	0.16	0.16	0.15
	Supplemented	0.53	0.48	0.46	0.42
Heterokaryotic fraction		0.20	0.18	0.20	0.20

as error, owing to differences in initial viability unrelated to the medium of origin. Because of these differences an average of the four controls was used in obtaining the surviving fraction of cells. Any error introduced by this procedure does not favor the hypothesis that there is a slow neutron effect: on the contrary, the apparent relative sensitivity of the boron-enriched cells would be slightly greater if their corresponding controls were used. The survival of the boron-free material is consistently higher than that of the B^{10}-enriched. The expected N^{15} effect is not seen, however.

To obtain a rough quantitative estimate of the expected effects, conidia from the same media of origin were exposed in the thermal column of the Oak Ridge graphite reactor. Fig. 3 shows their survival on minimal plating medium, relative to the thermal neutron flux. If, in the material exposed at the detonation, effects due to the boron n,α reaction are assumed to be essentially independent of those produced by the other radiations, an estimate can be made of the boron n,α effects alone. Accordingly, for each estimated dose, the survival of B^{10} conidia on minimal medium was divided by the survival of the corresponding boron-free conidia. Where two such ratios were available the values were averaged. The fluxes necessary to produce these calculated survival values in B^{10} conidia were estimated from fig. 3. Table 3 shows the comparison of these estimates with fluxes measured in the same hemispheres by the activation of gold. The agreement is surprisingly good, considering the crudity of the procedure, and the fact that the Neurospora estimates were made in ignorance of the gold fluxes. It becomes obvious from fig. 3 that the N^{15} effect could not be detected at these low fluxes.

SURVIVAL AND MUTATION OF CELLS EXPOSED IN A NITROGEN ATMOSPHERE

The protective effect of anoxia during irradiation has been shown in a wide variety of biological objects, including fungi (Stapleton and Hollaender, 1952) and, in general, decreases both the lethal and mutagenic effectiveness of ionizing radiations. With densely ionizing radiations the effect is very small, but with X-rays or gamma rays large effects are observed (see review by Patt, 1953). It is to be expected then, that with predominantly fast neutron radiation the protective effect of anoxia would

TABLE 3

THERMAL NEUTRON DETECTION WITH BORON ENRICHED CONIDIA

Estimated survival of boron n, α effect alone	Flux estimated from survival, thermal neutrons per cm^2	Gold flux
0.35	1.6×10^{13}	2.15×10^{13}
0.33	1.7×10^{13}	1.30×10^{13}
0.57	9×10^{12}	7.22×10^{12}
0.69	6×10^{12}	4.09×10^{12}
0.78	4×10^{12}	1.07×10^{12}

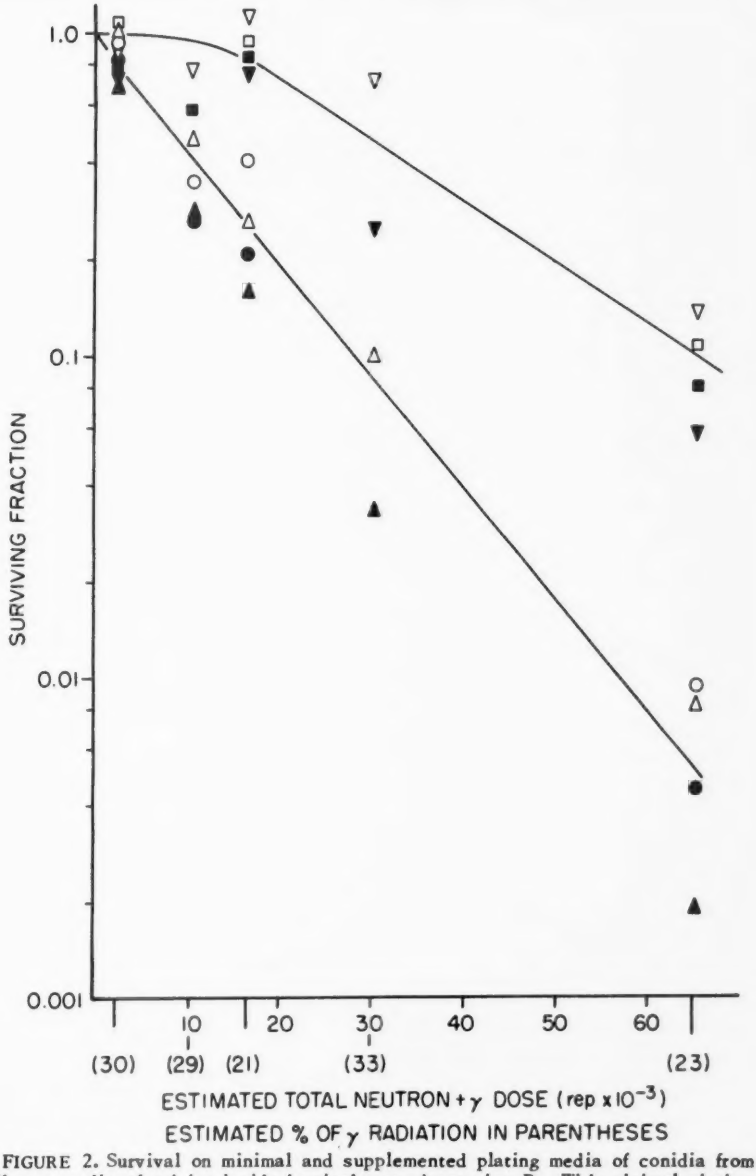

FIGURE 2. Survival on minimal and supplemented plating media of conidia from different media of origin; inside hemispheres, detonation B. With minimal plating medium the points indicate media of origin as follows: △ = N^{15}; ○ = Normal; ▲ = $N^{15}B^{10}$; ● = B^{10}. With supplemented plating medium: ▽ = N^{15}; □ = Normal; ▼ = $N^{15}B^{10}$; ■ = B^{10}. The curves conform in shape to those obtained with X-rays, approximating equation (1), upper curve for supplemented and lower curve for minimal plating medium.

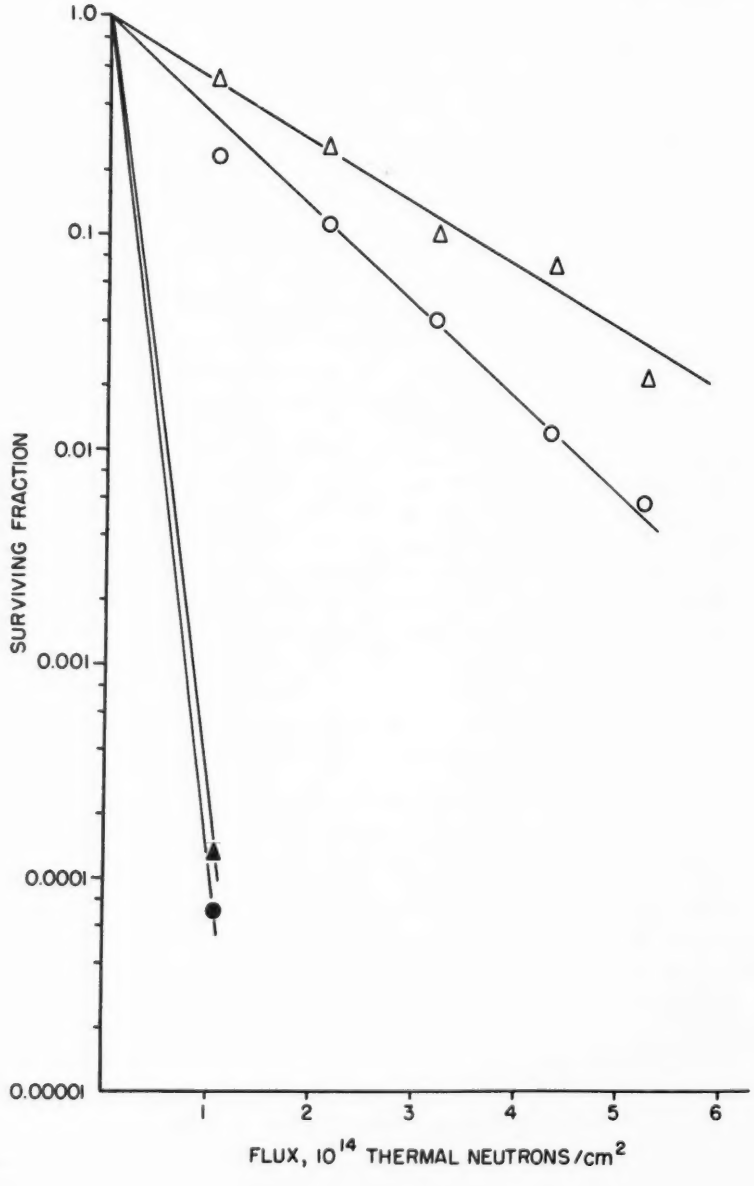

FIGURE 3. Survival on minimal medium of conidia from different media of origin, exposed in the thermal column of the Oak Ridge graphite reactor. The key to the media of origin is the same as in fig. 2.

TABLE 4

CONIDIA EXPOSED IN N_2 ATMOSPHERE, INSIDE HEMISPHERES

The comparable data for exposure in air are given in parentheses

Survival		Heterokaryotic fraction	Number of isolates tested	Percentage recessive lethals
Minimal	Supplemented			
		Detonation A		
0.09 (0.15)	0.54 (0.55)	0.12 (0.15)	112 (1425)	18.8 (13.5)
		Detonation B		
ca. 3.7×10^{-4} (0.033)	0.014 (0.25)	0.017 (0.084)	22 (228)	31.8 (9.6)

be small. Table 4 shows data obtained from exposures to detonations A and B made in an atmosphere of nitrogen. In detonation A all effects are judged to be the same as in air, within the limits of error. In detonation B, however, the effects in nitrogen appear to be greater than in air. Even with the low number of 22 isolates (because of the unforeseen low survival, no more were available) the yield of recessive lethals was significantly greater in nitrogen ($P < 0.05$). The survival and the heterokaryotic fraction were both decreased, suggesting that the apparently greater sensitivity in nitrogen is partially real, but the relation between the survival and the heterokaryotic fraction is anomalous, as shown in fig. 1. At least it can be stated with certainty that nitrogen afforded no significant protection, and the question of whether an anomalous sensitization was produced remains open.

SURVIVAL OUTSIDE THE NEUTRON HEMISPHERES

The principal difference to be expected between the mixed radiation outside and inside the biomedical hemispheres is that the radiation outside the 7-inch thickness of lead should comprise a much greater proportion of gamma rays. The total doses and distributions of these radiations are, of course, unknown. The survival of conidia exposed outside four of the hemispheres is shown in table 5. The estimated total rep inside the corresponding hemispheres is given for comparison. In many cases no survivors were found, but since the total number of cells plated is known, maximal limits for survival can be given. Unfortunately the outside samples exposed in a nitrogen atmosphere, which might have shown a protective effect consistent with the expected large gamma-ray component, were in a location where the survival could not be measured. Without knowledge of the relative biological effectiveness (RBE), the outside and inside effects cannot properly be compared, but if probable differences in RBE are ignored, it would appear that the lethal effects outside correspond to doses from 4.5 to 6.0 times greater than the inside doses. It is not

TABLE 5

SURVIVAL OF CONIDIA EXPOSED OUTSIDE NEUTRON HEMISPHERES, DETONATION B

Estimated total dose inside the corresponding hemispheres (rep) →			65,000		30,000		10,600		2,280	
Plating medium →			Minimal	Supplemented	Minimal	Supplemented	Minimal	Supplemented	Minimal	Supplemented
Medium of origin	Upper containers	N^{15}	$< 2 \times 10^{-7}$	1.1×10^{-5}	$< 8 \times 10^{-7}$	6×10^{-5}	0.022	0.16	0.52	1.07
		Normal	$< 2 \times 10^{-7}$	ca. 8×10^{-7}	1.4×10^{-5}	0.0044	0.0090	0.12	0.43	1.14
		$N^{15}B^{10}$	$< 3 \times 10^{-7}$	$< 5 \times 10^{-7}$	$< 9 \times 10^{-7}$	$< 6 \times 10^{-7}$	0.0054*	0.044	0.50	0.89
		B^{10}	$< 3 \times 10^{-7}$	$< 6 \times 10^{-7}$	$< 6 \times 10^{-7}$	$< 10^{-7}$	0.0018	0.046	0.22	0.44
	Lower containers	N^{15}					0.057	0.43	0.38	0.70
		Normal					0.030	0.26	0.34	1.20
		$N^{15}B^{10}$					0.0017*	0.020	0.27	0.94
		B^{10}					0.047	0.11	0.41	1.01

*In these instances the survival is in disagreement with the heterokaryotic fraction (see fig. 1).

certain whether the medium of origin is influential, because the effect may have been obscured by variability due to other, unknown causes. Where the survival could be measured the agreement between samples in the upper and lower containers was poor, although these were from the same medium of origin. In two cases (shown in fig. 1) the survival was much too low to be compatible with the heterokaryotic fraction, suggesting injury from causes other than radiation alone. It seems unlikely that excessive heating was a factor, since the thin paraffin coating at the ends of the containers showed no evidence of melting, but it is conceivable that mechanical shock played some role.

COMPARISON WITH LABORATORY RADIATION SOURCES

When the effects of the nuclear devices on survival are compared with those of X-rays, the RBE ranges between 1.5 and 5.0 depending on the medium of origin, the plating medium, and (in the case of supplemented plating medium) on the survival itself. When the comparison is restricted to the most suitable criterion, survival on minimal plating medium, the differences are less marked. Fig. 4 shows this comparison between Co^{60} gamma radiation, 250 kvp X-rays with 3 mm of Al filtration, fast neutrons produced with the Oak Ridge 86-inch cyclotron by proton bombardment of beryllium, detonation A, and detonation B. The data from detonation B are for the N^{15} boron-free medium of origin, whereas in detonation A, where no significant differences could be attributed to the medium of origin, all the data are used. With the cyclotron, no effect of the medium of origin was detected, although it is possible that differences would be revealed by longer exposures, since the energy distribution of the neutrons within the cyclotron facility is thought to be quasiexponential, resembling that in the neutron hemispheres. It was found, as expected, that the medium of origin did not affect the sensitivity of the conidia to X-rays.

From the data in fig. 4, the RBE of cyclotron neutrons and of mixed radiation from detonation B appears to be about 1.5 relative to X-rays and 2.0 to gamma rays, somewhat lower than in other biological materials. The RBE may be in error by a factor of 2 or more, because of errors in dose estimation. The somewhat greater effectiveness of radiation from detonation A may well be erroneous. Quantitatively, the experiments involving the nuclear devices are necessarily rather crude. The neutron dose estimates are based on extrapolation from measurements made with tissue equivalent dosimeters located within a suitable dose range, on the assumption that the total rep remains proportional to the component of neutron radiation measurable by the activation of sulfur. It is not known whether this assumption is valid. The gamma radiation was estimated with film badges. Surviving fractions of conidia at different estimated doses have greater internal consistency when related to the estimated total rep than to the neutron rep alone. This may be due in part to the rather small difference between the RBE of neutron and gamma radiations for the survival of heterokaryotic conidia on minimal plating medium, and perhaps to underestima-

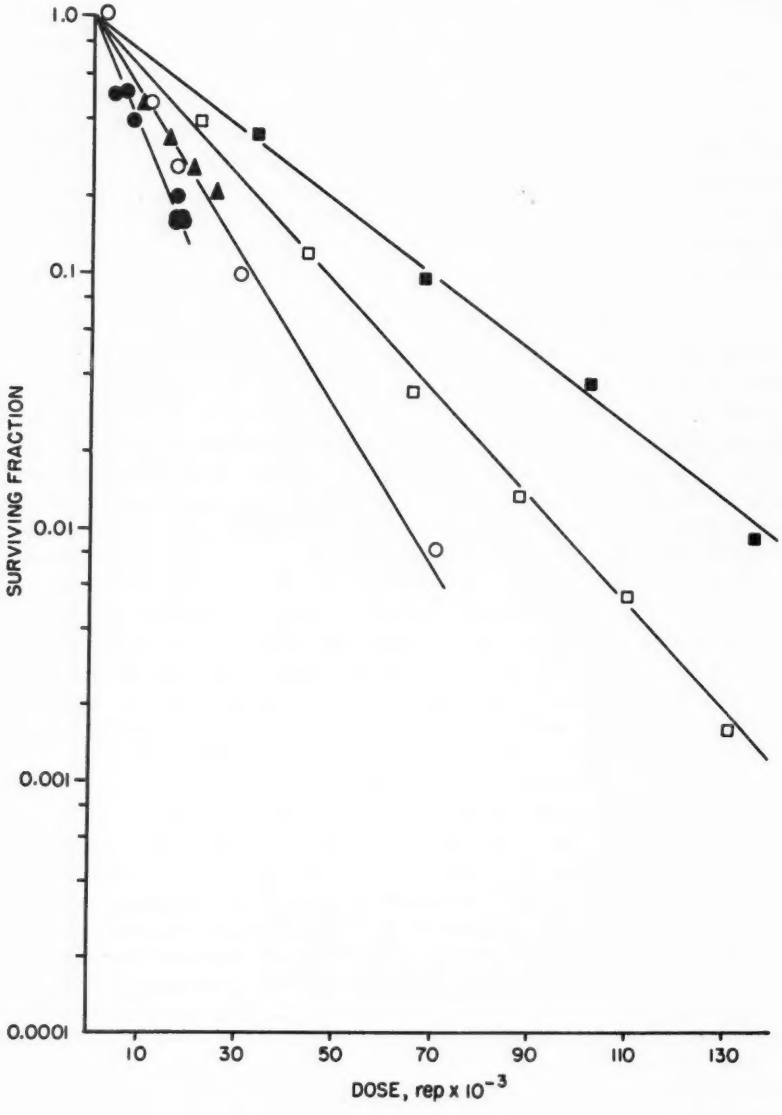

FIGURE 4. Survival on minimal medium with various radiations. ■ = Co^{60} gamma rays; □ = 250 kvp X-rays; ▲ = cyclotron neutrons; ○ = detonation B; ● = detonation A.

tion of the neutron dose. It is worth pointing out that we do not yet know whether the RBE of mixed radiation can always be computed with confidence from the RBE of the separate components. Granted these uncertainties, the internal consistency of the survival curves and the fair agreement between the cyclotron and detonation data suggest that the data may have somewhat greater validity than could justifiably be supposed from the nature of the physical estimates.

Similar comparisons were made with respect to recessive lethal mutation. The percentages of nuclei carrying recessive lethal mutations after treatment with various estimated doses in detonations A and B, and with X-rays, gamma rays, and cyclotron neutrons are shown in fig. 5. The points are bounded by 95 per cent confidence limits. Since no significant differences were found relative to the medium of origin, the data for the various media have been averaged for each estimated dose in detonations A and B. It is curious that there is not much difference in recessive lethal incidence between inside and outside stations in detonation B, even where there is a large difference in survival, a finding which we cannot yet fully explain. The apparently greater effectiveness of detonation A in decreasing survival is paralleled by a significantly higher recessive lethal incidence at the highest dose. Normally, the recessive lethal frequency in laboratory radiation experiments rises to a saturation value after which it remains constant, or in some instances decreases. The maximum values reached in different experiments are variable, and the causes of the variation are not yet known. In the presence of such variability precise comparisons of the

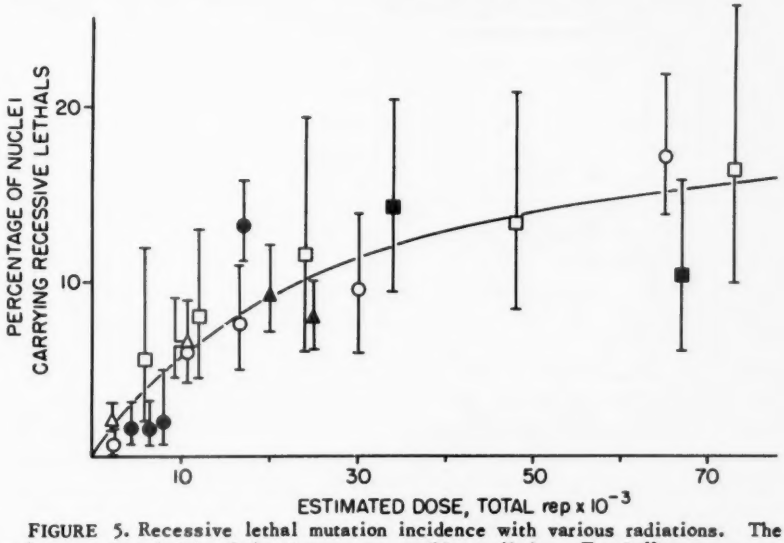

FIGURE 5. Recessive lethal mutation incidence with various radiations. The points are bounded by their 95 per cent confidence limits. ■ = Co^{60} gamma rays; □ = 250 kvp X-rays; ▲ = cyclotron neutrons; ● = detonation A; ○ = detonation B; △ = detonation B, outside hemispheres.

effects of different radiations are not possible, but it is clear that the results with the nuclear devices fit in well with the general picture.

DISCUSSION AND CONCLUSION

Perhaps the only unique feature of the radiation from a nuclear detonation is the fact that the dose (at least so far as neutrons are concerned) is delivered practically instantaneously, within a few microseconds. The question of whether the enormous dose rates would alter the biological effects of the radiations has often been raised, although admittedly with slight justification. Within the experience of the radiation biologist, the instances in which the dose rate may influence a biological effect are confined to those in which there is enough time for biological changes to take place before the delivery of the dose is completed. In situations where some manifold constituent of an organism must be exhausted, or brought to a threshold, by radiation damage, it follows that when the rate of inactivation of the constituents is low enough to be partially balanced by their replacement rate, the radiation will appear to be less effective than it would at higher intensities. The dose rates obtained with laboratory facilities can be made high enough to eliminate intensity effects brought about in this way, and the likelihood that a new cause for failure of reciprocity, or any new qualitative effect, might be found at much higher intensities seems remote. At extremely high intensities the interactions among the free radicals produced by different ionizing particles might significantly compete with the reactions between the radiation products and the biological material; that is, a saturation effect might ensue. However, it may readily be surmised that this condition would not ordinarily be detected biologically. For instance, it has been shown for α particles (Lea, 1947) that even in pure water the free radicals are lost through recombination within a few radii of the initial column, and these calculations would seem to be applicable to the densely ionizing recoil protons produced by fast neutron radiation. Thus the paths of the ionizing particles would have to be very close together before significant interaction would be obtained among radicals produced by different particles. Where the ionizations are dispersed, as with gamma radiation, the radicals would diffuse relatively long distances in water, but in biological material, where reactive solutes are present, the average distance that the radiation products diffuse from their points of origin before being removed from the system would be much less than in water. Assuming instantaneous dose; i.e., infinite intensity, rough computations based on tabulated data of Lea (1947a) for energy absorption in tissue and for ionic density of various radiations indicate that saturation effects are not to be expected until instantaneous doses of 10^5 to 10^6 rep are reached, a dose range within which there are few, if any, biological criteria by which the effect could be recognized.

The findings in Neurospora are in accord with the expected quality of the radiation. That it comprises predominantly fast neutrons is reflected in the better agreement of the results with those obtained with cyclotron

neutrons than with other radiations, the failure of anoxia to protect, and the small magnitude of the biologically measured slow neutron component. The slow neutron component measured with boron-enriched conidia is close to the physical estimates, signifying independence of the effects, even at high intensities. The few anomalous findings are isolated instances which can, in part, be explained, and are not for the present to be regarded as uniquely associated with the radiation from nuclear devices. The cells receiving an estimated 65,000 rep in detonation B were certainly subject to a dose rate equal to the highest ever achieved in a biological experiment. If the estimated 50,000 rep due to neutrons was delivered in, conservatively, 5 microseconds, then the dose rate was of the order of 10 billion rep per second. Yet by every criterion examined—the survival of the cells, the reduction in the heterokaryotic fraction, and the incidence of recessive lethal mutation, there is nothing to suggest any anomalous response due to high intensity. Despite the opportunities for error, the findings on Neurospora conidia, taken as a whole, strongly indicate that the effects of radiation from the nuclear devices are similar in all respects to those which can be produced in the laboratory.

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the cooperation of Dr. H. H. Plough of the Division of Biology and Medicine, U. S. Atomic Energy Commission; the data for dose estimates provided by Drs. C. W. Sheppard and E. B. Darden of Oak Ridge National Laboratory, by Dr. E. Tochilin and others of the U. S. Naval Radiological Defense Laboratory and by Drs. R. Butenhoff and L. J. Deal of the Radiation Instruments Branch, Division of Biology and Medicine, U. S. Atomic Energy Commission; helpful discussions with Dr. Sheppard, and the patience and competence of Dr. Robert Carter, lieut., USNR, to whom the success of the field experiments must be largely attributed.

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PUBLICATIONS RECEIVED

THE AMERICAN NATURALIST is glad to acknowledge here the receipt of books on biological and natural history subjects which are likely to be of interest to our readers. No undertaking to publish reviews is implied in this acknowledgment. Books for notice may be sent to:

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The American Naturalist
Box 2, Schermerhorn Hall
Columbia University
New York 27, N. Y.

Abbott, R. Tucker, 1954. American seashells. 541 p., 100 text-figures by Frederick M. Bayer, 40 plates in color. $12.50. D. Van Nostrand Company, Inc., New York.

Abnormal and pathological plant growth. Brookhaven symposia in biology No. 6. 303 p., ill. $2.10. Office of Technical Services, Department of Commerce, Washington 25, D. C.

Berndt, Ronald M., and Catherine H. Berndt, 1954. The first Australians. 144 p., illustrated. $4.75. Philosophical Library, New York.

Boell, Edgar J. (Editor), 1954. Dynamics of growth processes. 304 p., ill. $7.50. Princeton University Press, Princeton, N. J.

Papers given at the annual symposium sponsored by the Society for the Study of Development and Growth have in the past appeared as supplements to the journal *Growth*. As a departure from previous procedure, they appear here for the first time in book form. At the 1952 symposium thirteen papers were presented covering different aspects of animal and plants growth, and ranging in scope from biochemical synthesis and virus reproduction to hereditary mechanisms and population growth. The papers were presented in the following order: I. Virus reproduction and the replication of protoplasmic units (L. M. Kozloff); II. Experiments with the chemostat on the rates of amino acid synthesis in bacteria (A. Novick and L. Szilard); III. Cytochemical aspects of protein synthesis (A. W. Pollister); IV. Interaction of nucleus and sytoplasm in cell growth (G. Fankhauser); V. Cell and tissue differentiation in relation to growth (animals) (K. R. Porter); VI. Cell and tissue differentiation in relation to growth (plants) (D. S. van Fleet); VII. Physical factors affecting growth in plants (F. W. Went); VIII. Chemical regulation of growth in plants (F. Skoog); IX. Chemical control of growth in animals (R. Gaunt); X. The relationship of skeletal status to the physical growth and development of

children (W. W. Greulich); XI. Regularities in growth curves, including rhythms and allometry (D. A. Sholl); XII. Hereditary mechanisms in animal growth (G. E. Dickerson); XIII. Quantitative aspects of population growth (F. E. Smith).

The organized phenomena of growth and differentiation are very slightly understood, and the majority of the themes presented here are general and had to be subjected to some limitations. The methods chosen by the different speakers of coping with this difficulty and of integrating particular aspects and phases of research with the general picture of growth are most interesting and make the study of this volume a valuable experience.

ROBERT BLOCH

Bragg, Sir William, 1954. Concerning the nature of things. 232 p., 138 illustrations, $2.75 cloth; $1.25 paper. Dover Publications, Inc., New York.

Bridgman, P. W., 1954. The task before us. 112 p. 50¢. Proceedings of the American Academy of Arts and Sciences, Vol. 83, No. 3., Boston, Mass.

Briggs, John C., 1953. The behavior and reproduction of salmonid fishes in a small coastal stream. Fish Bulletin No. 94, 62 p. California State Fisheries Laboratory, San Pedro.

Condry, William, 1954. Thoreau. 114 p. $3.50. Philosophical Library, New York.

Darlington, C. D., 1954. The place of botany in the life of a university. 23 p. 40¢. Oxford University Press, New York.

Einstein, Albert, 1954. Essays in science. 114 p. $2.75. Philosophical Library, New York.

An abridged edition, non-scientific essays omitted, of Einstein's "The World as I See It," first published in 1933.

Emerson, Fred W., 1954. Basic botany. 425 p., ill. $5.00. The Blakiston Company, Inc., New York.

English, Horace B., 1954. The historical roots of learning theory. 21 p., $.65 paper. Doubleday Papers in Psychology. Doubleday & Co., Inc., Garden City.

Featherly, H. I., 1954. Taxonomic terminology of the higher plants. 166 p. $3.75. Iowa State College Press, Ames.

About one half of the book is given over to a glossary of botanical technical terms which is ample though it is not intended to be exhaustive. For those desiring quick information as to a term needed for a particular

description, there is another section in which terms are arranged according to subject. To aid in the understanding of the meaning of scientific names, a section listing specific epithets and their meanings has been added. Finally for those students of botany who have studied neither Greek nor Latin more than 600 word components are listed which are derived from Greek or Latin. For each one the meaning is given together with a word in which it is used. A concise and useful volume.

ROBERT BLOCH

Fisher, Ed. L., 1954. Marine tropicals. 56 p., ill. $1.50 (paper). Sub-Marine Studios, Miami, Florida.

French bibliographical digest—biology, botany, zoology, 1953 (December, No. 9, Series II). 96 p., gratis. Cultural Division of the French Embassy, New York, N. Y.

Gray, Peter, 1954. The microtomist's formulary and guide. 794 p., ill. $10.50. The Blakiston Company, Inc., New York.

Himes, Joseph S., 1954. Social planning in American society. Doubleday short studies in sociology. 59 p. 95¢ (paper). Doubleday and Company, New York.

Langlois, Thomas H., 1954. The western end of Lake Erie and its ecology. 479 p., 72 figures, $10.00. J. W. Edwards, Publisher, Inc., Ann Arbor, Michigan.

Larsson, Tage, and Torsten Sjögren, 1954. A methodological, psychiatric and statistical study of a large Swedish rural population. Supplementum 89, Acta Psychiatrica et Neurologica Scandinavica. 250 p. 25 Swedish crowns. Ejnar Munksgaard, Copenhagen.

Loomis, W. E. (Editor), 1953. Growth and differentiation in plants. A monograph of the American Society of Plant Physiologists. 458 p., ill. $7.50. Iowa State College Press, Ames.

Progress made in the last decade within the field of plant growth and morphogenesis has been very encouraging, and authoritative and diversified information is a matter of no small importance. The present volume offers a number of review articles combined with original research approximately up to 1951–1953. The student of theoretical and applied plant physiology and the general biologist will find the incorporation of historical material and the interpretation of recent advances equally useful. There are 18 chapters, 16 of which have valuable bibliographies. While two chapters are of a summarizing or general nature (Loomis, Sinnott), the rest range over a wide area of botanical interest from physiology proper to developmental morphology and differentiation. R. E. Buchanan discusses some elementary mathematics of plant growth; W. E. Loomis, various kinds and physiological aspects of growth correlations;

H. F. Rosene and E. J. Lund, bioelectrical fields and correlation; S. A. Gordon, the physiology of hormone action. H. S. McKee reviews structure and synthesis of protoplasm; G. F. Sprague, heterosis; N. C. Thornton, dormancy; A. W. Naylor, reactions of plants to photoperiod; H. C. Thompson, vernalization of growing plants; J. Bonner and J. Liverman, hormonal control of flower initiation. G. Baldovinos de la Pena reviews growth of the root tip; G. C. O'Kelley and P. W. Carr, elongation of the cotton fiber; B. Commoner and M. L. Zucker offer an interpretation of the phenomenon of cellular differentiation based on new observations and experimental work. K. Esau, in her article on anatomical differentiation in shoot and root axes, places emphasis on the vascular system. Factors associated with diseased growth are discussed by A. J. Riker and A. C. Hildebrandt; and comparative physiology of heterotrophic growth in plants, by S. H. Hutner. There are tables, graphs, illustrations, and a subject index.

ROBERT BLOCH

Magee, John L., Martin D. Kamen, and Robert L. Platzman, editors, 1953. Physical and chemical aspects of basis mechanisms in radiobiology. Publication 305, Subcommittee on radiobiology, committee on nuclear science, National Research Council, Washington, D. C.

Mandelker, Jakob, 1954. Matter energy mechanics. 73 p. $3.75. Philosophical Library, New York.

Minnaert, M., 1954. The nature of light and colour in the open air. 362 p., 16 plates, 160 text figures, $3.95 cloth; $1.95 paper. Dover Publications, Inc., New York.

Neider, Charles, Editor, 1954. The fabulous insects. 278 p. $3.50. Harper and Brothers, New York.

This anthology contains twenty-four selections on insects, spiders and scorpions. Mostly they were first published as magazine articles, but there are selections from books by Fabre, Maeterlinck, Beebe, Peattie, Curran and Sharp. Eleven of the selections are on social insects, five on ants and the others on bees, termites and wasps. The criterion of selection was that the pieces be "exciting" writing, but there is no flagrant nature-faking; indeed, for the most part, these are excellent examples of good popular writing about insects.

Nilsson, Heribert, 1953. Synthetische Artbildung. 2 vols., 1303 p. Paper 225, linen 250 Skr. Verlag C. W. K. Gleerup, Lund.

Ninth report of the Quebec Biological Bureau for the period November 1, 1950 to April 1, 1952. 521 p. Province of Quebec, Game and Fisheries Department, Montréal.

Payne-Gaposchkin, Cecilia, 1954. Introduction to astronomy. 508 p., ill. $6.00. Prentice-Hall, Inc., New York.

Pinner, Erna, 1953. Curious creatures. 256 p. Illustrated by the author. $4.75. Philosophical Library, New York.

This is a curious book. There is a striking drawing on almost every page—sometimes more impressionistic than accurate—accompanied by a breathless text on bizarre creatures from all sections of the animal kingdom and all parts of the world. It is arranged in chapters on topics like "The Struggle for Food," "Nursing Fathers," "What Comes out of an Egg," "Living Upside Down," "Birds that Cannot Fly." Things that may be true are generally stated as definite facts, which will cause raised eyebrows in scientific readers. I am not sure at what audience the book is aimed; perhaps the adolescent naturalist.

MARSTON BATES

Rand, Austin L., 1954. Social feeding behavior of birds. 71 p., $1.00. Fieldiana: Zoology, Vol. 36, No. 1, Chicago Natural History Museum, Chicago, Illinois.

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Mosbech, Johannes. Heredity in pernicious anaemia. A proband study of the heredity and the relationship to cancer of the stomach. Volume 34. 108 p. 23 tables. 5 pedigrees.

Øster, Jacob. Mongolism. A clinicogenealogical investigation comprising 526 mongols living on Seeland and neighboring islands in Denmark. Volume 32. 206 p. 42 ill. Numerous pedigrees and tables.

Sørensen, Hans Rahbek. Hypospadias. With special reference to aetiology. Volume 31. 94 p. 6 ill. Numerous pedigrees and tables.

Varia. Volume 30. 406 p. 42 ill.

University of South Carolina Publications, Biology, Series III, Vol. 1, No. 2, February, 1954. An Ecological Study of the Land Plants and Cold Blooded Vertebrates of the Savannah River Project Area.

Introduction (con't)—The Allendale Corridor, by William E. Hoy.

Part II. Fishes of the Savannah River Project Area, by Harry W. Freeman.

Part III. Ecological Observations on the Freshwater Sponges of the Savannah River Project Area, by James T. Penny.

Part IV. Succession in Fields of the Savannah River Project Area. 1. The Floristic Composition of Upland Fields in the Third Year of Abandonment, by Robert N. Tulloch and Wade T. Batson.

Natural Radioactive Contents of the Environs of the Savannah River Project, by W. C. Reinig, R. C. Williams, R. E. Gosline and E. L. Albenesius.

Research Committee, University of South Carolina, Columbia, S.C.

Watkins, Harold, 1954. Time counts. The story of the calendar. Foreword by Lord Merthyr. 274 p. $4.75. Philosophical Library, New York.

Weisz, Paul B., 1954. Biology. 680 p. ill. $6.50. McGraw-Hill Book Company, Inc., New York.

Zimmermann, Walter, 1953. Evolution. Geschichte ihrer Probleme und Erkenntnisse. 624 p. ill. DM 32. Verlag Karl Alber, Freiburg/München.

A study of the history of evolutionary thought from the vantage point of modern understanding of evolution is surely needed. Zimmerman's book is a valuable anthology of evolutionary writings, with informative and thoughtful commentary by the author. The period from the seventeenth century to Darwin is covered in most detail. The account of the post-Darwinian developments is brief and desultory but sound as far as it goes.

T. D.